PLASMA LEVELS OF INSULIN, GLUCAGON, AND PANCREATIC POLYPEPTIDE IN RELATION TO ADIPOSITY IN GENETICALLY SELECTED FAT AND LEAN CHICKENS.

by

Hugh Douglas Dimock

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Department of Animal Science
Macdonald College of McGill University

and

McGill Nutrition and
Food Science Centre
Royal Victoria Hospital

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suggested short title: The endocrine pancreas and adiposity in chickens.

This thesis is dedicated in memory of my late grandparents and to my family for all their love and patience through the years.

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ABSTRACT

HUGH DOUGLAS DIMOCK M.SC. ANIMAL SCIENCE

A genetically fat line (FL) of chicken has a lower glycemia than a lean line (LL). Four and five week old chicks were subjected to different states (fed, fasted 16 hr., and fasted then re-fed), ambient temperatures (5°C and 24°C) and diets in 3 experiments. Fasting reduced glycemia by 40-50 mg% versus fed levels and by 65 mg% versus re-fed levels and, while plasma insulin (IRI) levels decreased, glucagon (IRG) increases were inconsistent. Plasma avian pancreatic polypeptide (APP) levels were highest in re-fed birds (6 times fasted levels) versus fed (2-3 times fasted levels). Though hypothesized to be lipogenic, APP and IRI levels were not elevated in the FL. In one experiment, the IRG/IRI molar ratio was significantly higher in fasted LL versus FL birds suggesting an increased ability of LL birds to mobilize fat, as hypothesized. In response to cold, IRI, IRG, and IRG/IRI molar ratios changed in a direction consistent with conversion to a catabolic state. Dietary fat supplementation increased plasma glucose levels with no change in plasma IRI, IRG, or free fatty acids, suggesting a glucose sparing effect. Within lines, a rise in percent abdominal fat was correlated with a reduced glycemia in the LL only. Absolute

differences in IRI, IRG, or APP levels per se could not account for the "fattening" of the FL. IRI and IRG were not negatively correlated in the FL, suggesting an abnormal relationship. Equivalent IRI levels in both lines implied an increased sensitivity to IRI in the FL.

CONCENTRATIONS PLASMATIQUES DE L'INSULINE, DU GLUCAGON ET DE LA POLYPEPTIDE PANCREATIQUE EN RELATION AVEC L'ADIPOSITE ET LA SELECTION GENETIQUE DE POULETS GRAS OU MAIGRES

RESUME

HUGH DOUGLAS DIMOCK M.SC. ANIMAL SCIENCE

La glycémie est moindre chez des poulets d'une lignée grasse (LG) que chez ceux d'une lignée maigre (LM). Des poulets agés de 4 et 5 semaines ont été soumis à différentes situations (sans jeûne, jeûne de 16 heures, ou jeûne de 16 heures et renourris), à des variations de température (5°C et 24°C), et à diverses rations dans trois expériences. Les poulets à jeun avaient une glycémie inférieure de 40 à 50mg% à celle des poulets sans jeûne et, de 65 mg% à celle des poulets renourris, alors que leurs concentrations plasmatiques d'insuline (IIR) étaient moindres et que celles du glucagon (GIR) montraient des augmentations inconsistantes. Les poulets renourris avaient des concentrations plasmatiques de la polypeptide pancréatiques aviaire (PPA) trés supérieures (6 fois celles du jeûne) à celles des poulets sans jeûne (2-3 fois celles du jeûne). En dépit de leur potential lipogénique, les concentrations PPA et IIR n'étaient pas supérieures chez les poulets LG. Dans une expérience, la fraction molaire GIR/IIR à la fin du jeûne était plus grande chez les poulets LM que LG, indiquant une meilleure abilité des poulets LM à mobiliser leurs graisses, tel qu'anticipé. Le froid a amené des modifications dans IIR, GIR et les fractions molaires

GIR/IIR consistantes avec des activités cataboliques. Une ration enrichie de gras a causé une plus forte glycémie sans affecter IIR; GIR et les acides gras libres dans le plasma, suggérant une protection du glucose. À l'intérieur des lignées, seuls les poulets LM ont montré qu'une augmentation dans le pourcentage du gras abdominal était associeé avec une reduction glycemique. Les différences absolues dans IIR, GIR et PPA ne peuvent per se expliquer les mécanismes d'engraissement des poulets LG. Chez ceux-ci, il n'y avait pas une corrélations négative entre IIR et GIR, indice d'une interdependance anormale. Des concentrations d'IIR plasmatiques similaires chez les poulets des deux lignées impliquent une sensitivité accrue à IIR chez les poulets LG.

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GENERAL INTRODUCTION

Over the past 35 years or so the broiler industry has selected chickens demonstrating fast growth resulting in earlier marketability. Concommitantly, excess fat deposition has resulted. In an effort to understand the mechanisms of this fatness with a view to producing birds of lower fat content and more muscle in this same length of time, the mechanisms governing fat deposition i.e. intermediary metabolism and its regulation were studied.

A fat line (FL) and a lean line (LL) of chicken were developed, in France, by Bernard LeClercq and co-workers (1) by divergent selection from a mixed though common gene pool. Both lines grew at the same rate and consumed the same amount of feed yet the fat line deposited more fat than the lean line. Normally, when chickens are compared to mammalian species, they are hyperglycemic with plasma glucagon levels severalfold higher and plasma insulin levels several times lower. These are among the major determinants of fuel homeostasis, and thus could be implicated in differential deposition of fat versus protein. The FL was found to have a plasma insulin concentration which tended to be higher than the lean line (though nonsignificantly so) with a corresponding and significantly lower plasma glucose. It was thus of interest to investigate these and other parameters involved in intermediary metabolism in these experimental

lines. Glucagon, often said to be more important than insulin as a metabolic regulator in the bird, was chosen, as well as avian pancreatic polypeptide (APP) to further develop an hypothesis related to the development of fatness.

LITERATURE REVIEW

Introduction

This review will begin with a discussion of the present knowledge of the glucose-insulin relationship in the FL with respect to accounting for differences between the FL and LL birds. A brief discussion of glucoregulation and hormone-receptor binding as found in mammals, with appropriate cross-referencing to avian species where data are available, will follow. Next, a discussion of the anatomy and endocrine cell types of the avian pancreas will be presented. Then, a short description of the pancreatic endocrine hormones of interest, their structures, and modes of action and interaction will be alluded to. The major differences and similarities between birds and mammals in their control of intermediary metabolism will be discussed throughout. Finally, fat metabolism, as it is believed to be regulated in the FL and LL birds will be reviewed leading up to the hypotheses of this project.

Models of Fatness and the FL/LL

Mammalian models such as the Zucker rat and the ob/ob mouse are useful for the study of mammalian obesities. These rodents exhibit single gene autosomal-recessively inherited (monogenic) obesity (2). As complex as such monogenic obesity is, polygenic obesity, the type found in poultry, is even more complex (2). Thus the understanding of obesity in poultry requires avian models since one cannot extrapolate

from other experimental models. It is for this reason that the FL and LL of chicken were developed. Other fat and lean chicken models have also been studied (3,4). While the body fat content of the fat line used in this research is significantly greater than that of the lean line, a massively obese condition, such as found in mammalian models, does not exist. Rather, the FL might represent the type of bird used commercially in Canada and throughout the world. Diseases such as auto immune thyroiditis have been implicated in obesity in other avian models (5).

Development of these fat and lean birds began in Nouzilly, France at the Station de Recherches Avicoles, in 1976, when broilers were selected for divergent abdominal fat to body weight ratios (1). Abdominal fat was highly correlated with total carcass lipids (1,6). The program was designed to produce lean and fat birds with similar live weights at 63 days of age. Selection was based solely on the fattening of males. Changes in the ratio of abdominal fat to body weight in the two lines were rapid (1). The effect of selection on other adipose tissues was also studied. In 52-day-old male chickens the FL to LL ratio was 4.6 for abdominal fat, 2.6 for subcutaneous fat, 1.5 for adipose tissue located between muscles, and 1.6 for intramuscular total lipids (7).

Differences in body temperature or feed intake could not account for the FL depositing more fat. However, it was observed that the FL chickens showed a decrease in the

plasma glucose level soon after hatching in both the fed and fasted states. It was then observed that a glucose-insulin imbalance existed in the FL and this was used to partially explain the excess adiposity (8,9). This imbalance was observed after a glucose load that elicited a greater (sometimes three-fold) insulin release in the FL leading toward more lipogenesis relative to the LL. More specifically, at 6 and 8 weeks of age, glucose tolerance testing elicited a significantly higher insulin release in the FL versus LL chickens concommitant with a faster rate of decline of glycemia from peak values at 6 weeks of age in the FL but not at 8 weeks of age. An insulin "resistance" was suggested at this latter age in the FL (9). Indeed, it is known that obesity in man and in mammals is often associated with glucose intolerance and accompanying hyperinsulinemia. Obesity is a progressive condition comprising two main phases: an early one without insulin resistance, and a later one with apparent insulin resistance (10).

Three hypothetical mechanisms have been suggested to explain why the FL chickens do not become overtly obese in the long term: 1) genes of true obesity exist in the chicken but were not present in the initial selected flock, 2) other regulatory mechanisms develop and counteract the initial propensity or 3) insulin receptors, which are already largely depressed in chicken tissues, do not "down regulate"

in response to increased insulin levels after food ingestion so that no overt hyperinsulinemia develops in the FL chicks (11). The physiological situation of FL chickens appears similar to the short-lived "preobese" state observed in mammals (11).

Carbohydrate and lipid metabolism and their hormonal control in birds differ in some respects from those which operate in mammals (for reviews see 12,13,14,15). For example, in birds, basal glucose levels are twice those found in mammals. Before beginning a more detailed discussion of the bird it should be mentioned that most of our knowledge of avian carbohydrate and lipid metabolism is derived from studies using "domestic" avian forms such as the chicken, duck, goose, and to a lesser extent the pigeon. The understanding of many aspects of avian metabolism has lagged behind that of equivalent relationships in mammalian systems (12).

Much of the knowledge gained with respect to the pancreatic hormones in birds and their roles in metabolism has come from "artificial" experimental situations involving in vitro techniques, injection of pharmacological amounts of substances and surgical extirpation of the pancreas itself. The results have been valuable though at times confusing. They must be interpreted keeping in mind the experimental situations from whence they came. With this in mind an attempt will be made to provide an overview of the knowledge

to date in birds and the methods used to gain this knowledge.

Glucoregulation

A. Glucoregulation in the steady resting state

The brain requires a constant supply of fuel throughout life to ensure health and survival. There has thus evolved, in birds and mammals, a virtually foolproof system for maintaining cerebral fuel delivery to deal with rapid and unpredictable changes in the availability of cerebral fuels in the environment (16).

Except during meals and during extended fasting, production of glucose by the liver is the sole source of cerebral fuel. In a non-stressed normal subject, the basal glucose level will tend to remain the same (70-110 mg% (mg/dl in SI units) in humans; 180-240 mg% in birds) day after day because of the intrinsic feedback loop shown in figure 1 (17). For example, any tendency for the glucose concentration to increase is counterbalanced by an increase in insulin secretion and a suppression of glucagon secretion, which regulate hepatic glucose production and tissue glucose uptake to keep the plasma glucose concentration constant (17). If the subject gains weight or becomes insulin resistant, blood glucose levels may increase, resulting in increased insulin secretion to compensate for the insulin resistance. Thus, insulin and glucose levels are modulated

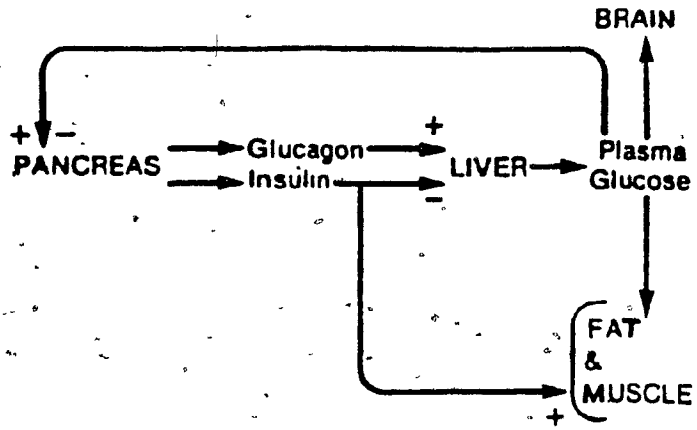


Figure 1 A model for steady-state regulation of plasma glucose. Plasma glucose has direct effects on the pancreas to increase insulin and decrease glucagon secretion during hyperglycemia, and to increase glucagon and decrease insulin secretion during hypoglycemia. Glucagon stimulates glucose production, and insulin suppresses glucose production from the liver and increases glucose uptake in the muscle and fat. Glucose uptake is not insulin-dependent in the brain. Any change in hormone secretion or hormone sensitivity will be modulated by this loop to minimize the change in glucose concentration and maintain peripheral glucose utilization. (From Pfeifer, M.A., J.B. Halter, and D. Porte, Jr., 1981. Insulin secretion in diabetes mellitus. Am. J. Med. 70:579.)

in an effort to minimize changes in these concentrations while relatively normal production and utilization of glucose are being maintained (17). While there are many influences tending to increase or to decrease plasma glucose levels, the end result of glucoregulation is not only to "avoid" hyper- and hypo-glycemia but to keep fluxes normal within a tightly regulated range.

Control over adaptation to fasting, in mammals, is exerted primarily by insulin and secondarily by the interaction of insulin and glucagon and with "permissive" effects by a wide variety of hormone modulators (18) (see table 1). Circulating glucose levels decline, and combined with other influences, serve to lower insulin levels (18). This decline results in an increase in adipose tissue lipolysis (with a resultant increase in plasma free fatty acids (FFA) and glycerol) and release of amino acids from muscle protein. The liver takes up more fatty acids and gluconeogenic substrate for energy supply, for ketogenesis and for gluconeogenesis. Glycogenolysis occurs in the liver to further supply glucose.

B. Glucoregulation during survival crises

Maintenance of cerebral glucose delivery during "fight or flight" emergency situations, requires instantaneous adjustments of the steady state equilibria that prevail in the resting and near-resting steady state. It is in these situations that, in terms of species survival, the most

A. **Primary regulators**
   - Insulin
   - Insulin-glucagon relationship

B. **Modulators**
   - **Pituitary**
     - Growth Hormone
     - ACTH, Prolactin, TSH
   - **Adrenal**
     - Glucocorticoids
     - Catecholamines
   - **Reproductive Organs**
     - Estrogens
     - Androgens
     - Placental lactogen
   - **Thyroid**
     - Throxine (T_4)
     - Triiodothyronine (T_3)
     - reverse triiodothyronine (rT_3)
   - **Other**
     - Somatomedins
     - Somatostatin
     - Growth factors
     - Gut hormones

TABLE 1 Humoral regulation of energy and protein homeostasis. (From Marliss, E.B., 1978. The physiology of fasting and semistarvation: roles of 'caloristat' and 'aminostat' mechanisms. In "Recent Advances in Obesity Research: II, Proceedings of the 2nd International Congress on Obesity," Bray, G.A. (ed.), Westport, Conn.: Food and Nutrition Press, Inc.)

important islet cell response is required (16). Since the energy stores within the exercising muscles (glycogen and lipids) are limited, exercise requires a substantial increase in glucose and free fatty acid production. Consequently, hepatic glucose production must constantly equal the total glucose used by the brain and by exercising muscle (16).

The major hormonal mediators of this hepatic response are the catecholamines and glucagon, their relative importance varying from species to species. Cortisol levels rise in exercise and may well potentiate and prolong the effects of glucagon and catecholamines on the liver (16).

At least two overlapping glucoregulatory systems exist in mammals to prevent and/or correct hypoglycemia: 1) the islets, which are the moment-to-moment regulators of glucose flux under basal and near basal steady state conditions, and 2) the sympathetic nervous system, which prevents hypoglycemia during exercise-induced glucose need. Both systems must be able to detect declines in plasma glucose and signal the liver to enhance glucose production and release (19). It is generally believed that similar systems operate in avian species.

The mediators of the sympathetic nervous system, the catecholamines, act both by glycogenolytic and gluconeogenic actions on the liver, via the islets, through alpha-adrenergic suppression of insulin and beta-adrenergic

stimulation of glucagon and by inhibiting insulin-mediated peripheral glucose utilization (19).

During exercise in mammals and birds, the sympathetic nervous system replaces glycemia as the primary controlling influence over the islets; alpha-adrenergic inhibition of insulin secretion (20) and beta-adrenergic stimulation of glucagon secretion (21) regulate the islet response in anticipation of, rather than in response to, increased glucose utilization (19).

C. Glucoregulation during meals

During alimentation, the role of the islets is to prevent excessive increases in the plasma levels of the ingested nutrients, in particular glucose. The response of the islets will vary with the composition of the meal. For example, a meal devoid of carbohydrate triggers a glucagon response to prevent hypoglycemia from the protein-induced insulin secretion (16). The ability of normal individuals to ingest large glucose loads with but a modest change in glycemia is due to the appropriately timed and quantitated release of insulin with the role of glucagon limited to determining the fraction of the ingested glucose stored in the liver (16).

Hormone-Receptor Binding

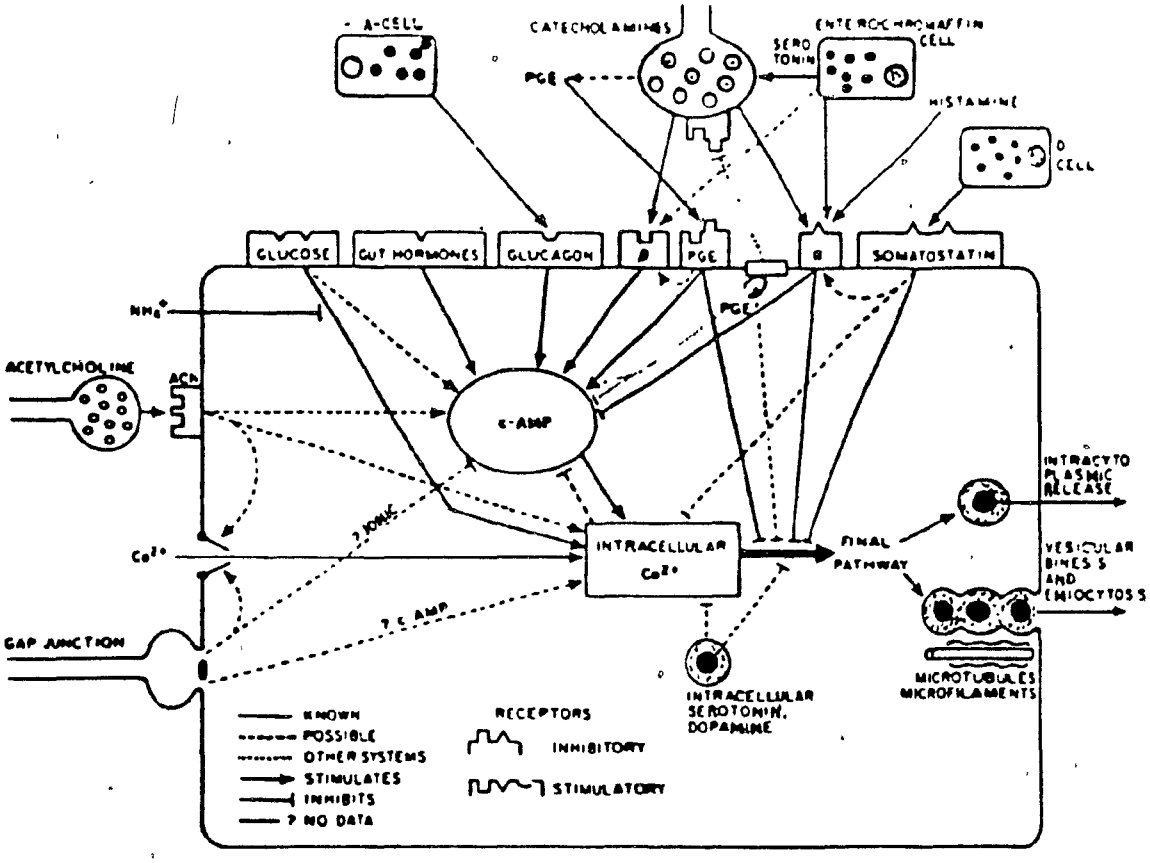
Functionally, hormone binding usually involves the

following: 1) the receptor sites bind the corresponding hormone with a high degree of affinity and specificity, 2) the specific binding sites are finite in number, 3) they are located, or predominate, on the cell plasma membrane, 4) hormone binding is rapid and reversible, and 5) hormone binding to these sites can be related to the biological effects of the hormone either directly or indirectly (22). It should be mentioned that some receptor sites can vary from having a high affinity for their respective hormone but a low binding capacity to others which have a low affinity coupled with a high capacity for hormone binding.

Hormone action on a target cell involves: 1) binding of the hormone to the receptor and formation of the hormone-receptor complex (HR), 2) "coupling" between (HR) and the effector(s) which results, via the formation of a second messenger, in the modification of a variety of events, e.g. stimulation of membrane transport systems, enzyme activation or inactivation and modification of protein synthesis (22).

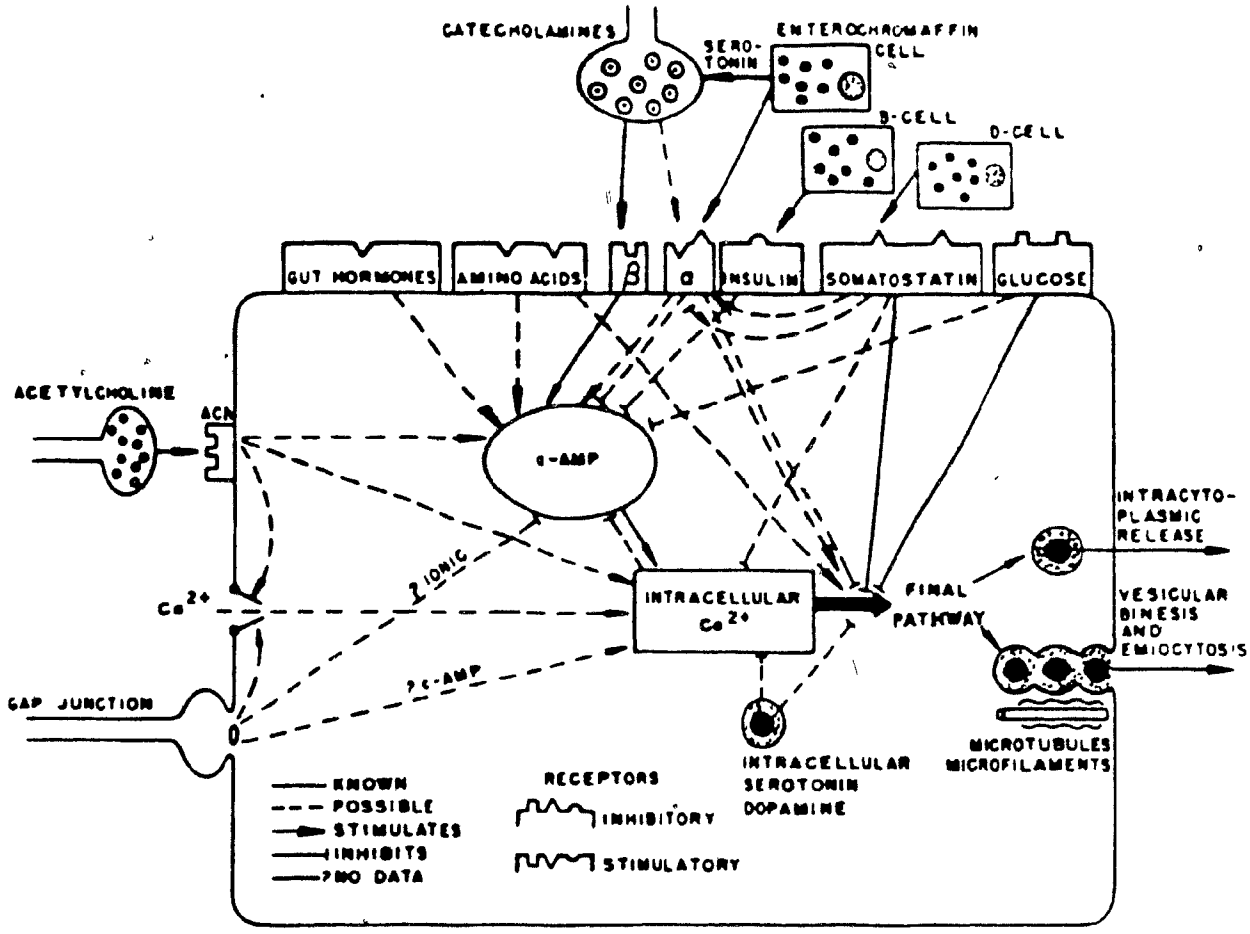
Hormone action is also dependent upon its concentration in the circulation and the status of the receptors i.e. whether the receptors are repressed or derepressed. Therefore, metabolism is regulated by hormone needs, stores, and the methods for controlling hormone flux. The ability of hormones to respond to stimuli, to either increase or decrease their concentration is vital for normal body metabolism. In addition there is a wide variety of post-

receptor events that determine hormone effectiveness. The events surrounding the pancreatic release of insulin and glucagon from B and A cells and the other factors controlling glycemia are too numerous to discuss but are summarized in figures 2 and 3 respectively.



INTERACTING SYSTEMS IN INSULIN SECRETION

Figure 2 Neuroendocrine regulation of the B-cell. A summary description of some of the factors impinging on the basic glucose control of insulin secretion. (From Smith, P.H., and Porte, D., Jr. 1976. Neuropharmacology of the pancreatic islet cells. Ann. Rev. Pharm. Toxicol. 16:269)



INTERACTING SYSTEMS IN GLUCAGON SECRETION

Figure 3 A schematic description of the neuroendocrine regulation of the A-cell. (From Porte, D., Jr., et al. 1976. Neurohumoral regulation of the pancreatic islet A and B cells. Metabolism 25:1453)

The Avian Pancreas

The avian pancreas is a discrete lobular structure located in the duodenal loop. It consists of three large lobes, the ventral, dorsal and "third" lobes. Extending in isthmus-like fashion from the "third" lobe is a very small splenic lobe (23,24). The endocrine pancreas is disposed throughout the exocrine component in discrete islets. There are 3 main and distinct islet types (based on distribution of the cell types found therein) which release glucagon, insulin, and somatostatin, and a fourth type that releases avian pancreatic polypeptide (APP) (14,25). Endocrine cell types include A (glucagon), B (insulin), D (somatostatin), and PP (pancreatic polypeptide, or "F") cells. The splenic lobe has a high concentration of A or D type cells. Regulation of avian endocrine pancreatic secretion is under intestinal hormone, nutritional, autonomic nervous system, and paracrine control (23). Pancreatic hormone-plasma metabolite feedback mechanisms, are the main regulators of these secretions (14). There is also an exocrine function to the avian pancreas.

The Pancreatic Hormones

A. Avian insulin

Avian insulin is synthesized by the pancreatic B-cell ribosomes as preproinsulin then proinsulin, a single-chain polypeptide of MW 9100 daltons. The A chain is connected to

the B chain by a biologically inert C chain (connecting peptide). After cleavage of the C peptide, the biologically active double-chain molecule possessing two disulfide cross-linking bonds, is condensed forming B-cell granules. The structure of chicken insulin is similar to that of most mammals with the A chain containing 21 amino acids and the B chain 30 amino acids (26,27). Chicken insulin differs in structure from its mammalian counterparts no more so than the latter do amongst themselves (26) but the biological effect of avian insulin in mammals is very different. This might be due to the fact that there are known binding domains and biological effect domains and immunoreactive domains and that one or more of the nonhomologous amino acids are at these critical places. Indeed, this has been studied by others (28).

The structure of turkey insulin is identical to that of chicken insulin whereas duck insulin differs from chicken insulin in the presence of glutamine and proline at positions 8 and 10, respectively, on the A chain and by threonine at position 30 on the B chain (29).

B. Avian glucagon

Proglucagon is produced and cleaved within the $alpha_2$ cells of the pancreas to produce glucagon (30,31); these $alpha_2$ cells reside in the so-called "dark islets", which also contain D cells (or $alpha_1$ cells in older texts). The glucagon content of the avian pancreas is at least four to

eight times greater than levels found in equivalent amounts of mammalian pancreata (24,32,13).

While all mammalian glucagon structures possess the same single chain amino acid sequence of 29 residues, three avian glucagon structures have been elucidated. Chicken and turkey glucagons have a substitution by serine for asparagine at position 28. This substitution provides the bird with an extremely potent hormone regardless of which vertebrate species it is tested upon. Duck glucagon is similar to chicken and turkey glucagon but has an additional substitution by threonine for serine at position 16 (33). These substitutions have been suggested to lessen favourable cross-reactivity in immunoassay systems (13).

Immunoreactive glucagon (IRG) or true pancreatic glucagon and glucagon-like immunoreactive glucagon (GLI) or "gut" glucagon, originating from the stomach and small intestine, are distinguished from each other based on their abilities to react with specific antibodies. Polypeptides that react with antisera directed against an immunodeterminant within the COOH-terminal (residues 24-29) of glucagon are called IRG. Those that react with antisera raised against an immunodeterminant within the NH_2-terminal and central (residues 2-23) regions of glucagon but not with COOH-terminally directed antisera are labelled GLI (34).

The physiological significance of GLI is unknown. Gut GLI's

may be a primitive family from which structurally related polypeptides such as glucagon, secretin, vasoactive intestinal peptide (VIP), and gastric inhibitory peptide (GIP) have evolved (34). It is also possible to get "true" IRG from the stomach, especially in depancreatized animals (Marliss, 1985 pers. comm.).

C. Avian Pancreatic Polypeptide-

APP, a double helical coiled polypeptide of 36 amino acid residues, is synthesized as a much larger (around 8000 dalton) polypeptide by endocrine type PP cells. The pro-molecule is cleaved of a 25-amino acid residue attached to the N-terminus (23). This hormone lacks disulfide linkages and possesses four proline, one isoleucine, and a cluster of basic residues near the exposed tyrosine amide C-terminus (28,35,36,37). There is a certain degree of homology between APP and glucagon structures (35,38).

Similar polypeptides of identical length have been found in bovine, ovine, porcine and human pancreata (39). Rat PP has been isolated and sequenced. Despite a high degree of homology, rat PP will hardly react with antibodies to human and bovine PP (40).

Modes of Action and Interaction of the Pancreatic Hormones

The avian liver is the main site of lipogenesis in the bird, deriving most of its required carbon skeletons from ingested fructose, glucose, galactose, and mannose (12). Adipose

tissues play a secondary role, in this respect, to the liver in avian carbohydrate and lipid metabolism. The pancreatic hormones exert their greatest impact on the liver followed by, in order of decreasing significance, skeletal muscle, adipose tissue, cardiac muscle, and the erythrocyte in the bird (12).

A. Insulin

Avian insulin circulates at variable levels depending upon species, time of day, state of nutrition, and degree of physical activity. A "normal" circulating level would be about 30-40 uU/ml (1-2 ng/ml) plasma in 4- to 6-week old chicks and 60-90 uU/ml plasma in 10- to 24-week old chickens (12). Duck normoglycemia is 50-70 mg% below that of chickens with plasma insulin levels one-half chicken levels (12). The half-life of chicken insulin in the circulation is 5-7 minutes (13).

Mechanisms of avian insulin release from islet cells are based on studies involving injection of "physiological" substances into chickens and ducks (12). Plasma insulin levels are relatively unaffected by progressive fasting (24). The chicken B cell is insensitive to all but very high concentrations of glucose and is incapable of a sustained increase in insulin release (41). Hyperglycemia caused by glucose injection doubles or triples insulin levels within 20 minutes in chickens. Induced hyperaminoacidemia has the

same effect. Both glucose and amino acids are more effective when given per os as in mammals (13). Glucagon injection elevates glucose and free fatty acid levels but, insulin release is unaffected (24). In contrast in ducks, glucagon infusion raises insulin levels disproportionately to the degree of hyperglycemia produced (42). Acetylcholine stimulates insulin, glucagon, and somatostatin secretion by the avian pancreas. In fact, regulation of the B cell by the cholinergic system may predominate over glucostatic control (43). It is believed that similar mechanisms are involved in controlling insulin regulation in the chicken and in mammals i.e. changes occur in the sensitivities of various tissues and the role of the entero-insular axis (44).

The drug tolbutamide is a potent hypoglycemic agent in chickens and ducks as well as in mammals. It induces a short-term insulin release rapidly and dose-dependently in chickens and mammals. It is thought that other factors potentiate the hypoglycemic effect of tolbutamide-induced insulin release (45). Alloxan and streptozotocin are not cytotoxic to chicken pancreatic B cells even at high sublethal doses (46) as opposed to the situation in mammals.

In isolated rat fat cells and liver plasma membranes, chicken insulin is more biologically potent than porcine insulin and this is accounted for by an increased binding affinity by chicken insulin for the insulin receptors (47). In rat adipocytes, chicken insulin is again more potent than

porcine insulin in stimulating glucose utilization and inhibiting glucagon-stimulated lipolysis (48).

Birds have been shown to be resistant to the hypoglycemic effect of exogenous avian insulin even at supra-physiological levels (12). Thus, the essentiality of insulin in the regulation of normal carbohydrate metabolism has been questioned. In chicken hepatocytes and thymocytes there are 3-5 times fewer insulin binding sites than in rat liver (11,49). This paucity of receptors may explain, in part, chicken insulin "resistance" (11,50) though very few receptors per cell must be occupied for full biological effect. But, resistance may be due to events occurring after the hormone-receptor interaction (51,52).

Insulin's ability to regulate plasma glucose is complex, involving synthesis and secretion of insulin, its transport in the bloodstream, and its action at the target cell level (53). Using perifused, non-collagenase digested "microfragments" of chicken pancreas it was shown that glucose may not modify insulin levels in the chicken or alternatively it may modify insulin levels only after endocrine or neural alteration of the sensitivity of the B cells to glucose (54). At the cell membrane insulin receptor site, insulin probably initiates cytoarchitectural changes which favour the intracellular translocation of glucose prior to its phosphorylation and subsequent metabolism (32,13). Insulin appears though to facilitate glucose

transport and adjust tissue sensitivity to the effects of other hormones (13).

The role of insulin in growing chickens is ambiguous. In chick embryos insulin stimulates glucose and amino acid transport, DNA and RNA synthesis and cell multiplication. In growing chickens though, lipogenesis in hepatocytes and glucose oxidation in adipocytes are stimulated only slightly by insulin (49). Insulin stimulates amino isobutyric acid uptake by increasing the influx with no effect on the efflux (49). By its effect on glucose metabolism, insulin helps to provide the energy for protein synthesis and may also directly affect amino acid uptake by muscle cells (55).

Birds that consume high carbohydrate, low fat seeds and other plant products, rely on glucose as a major precursor of lipid synthesis. Insulin, by its action on glucose transport, thus would appear to play a principal role in the regulation of lipogenesis. Insulin, to a lesser degree, influences triglyceride synthesis by regulating esterification through provision of substrate, and alpha-glycerol phosphate (12). These observations are of fundamental significance in our FL/LL studies with respect to the glucose-insulin imbalance in the FL.

Fatty acids are supplied by plasma fatty acids, by synthesis from glucose, and by hydrolysis of existing triglycerides. Insulin appears to directly stimulate lipoprotein lipase and thus regulates uptake of triglyceride fatty acids by

extrahepatic tissue (12). Contrary to general opinion, insulin appears to be anti-lipolytic, in birds, as in mammals (14). In ducks, insulin has a glucose-dependent hypolipacidemic effect (56). Thus, insulin can lower the plasma FFA level in the presence of pancreatic glucagon (56). Glucagon, epinephrine and other lipolytic hormones inhibit lipoprotein lipase activity (12).

Insulin favours hepatic glucose uptake and intracellularly it increases glucokinase and glycogen synthetase activity. It thus lowers blood glucose, resulting in increased hepatic synthesis and deposition of glycogen. As in mammals, insulin increases protein synthesis; gluconeogenesis is remarkably reduced as a result of decreased activity of the major gluconeogenic enzymes (12).

B. Pancreatic glucagon (IRG)

In normal ducks (42,57) and normal geese (56,59) plasma glucagon levels are 2 to 8 times higher than in mammals with corresponding pancreatic concentrations 4 to 8 times higher in birds (60). A greater importance of glucagon in regulating metabolism in birds is thus suggested. In addition to true pancreatic glucagon, there at least four other plasma components of IRG separated according to molecular size. These other forms, however, do not possess the biologic activity of true pancreatic glucagon which, by virtue of its molecular weight is designated IRG^{3500} (16).

It is assumed that avian glucagon acts at the periphery by interacting with membrane bound adenyl-cyclase. Its effects are usually rapid and transient, cyclyzing adenosine triphosphate (ATP) to form cyclic 3',5'-AMP, the "second messenger" of hormone action. The effects produced are dependent on the cell involved. They include hormone release (pancreas), Ca^{2+} and K^{+} release (liver), lipolysis (adipose and hepatic tissue), protein catabolism (liver) and glycogenolysis (liver)(55,61). Glucagon-induced lipolysis is generally considered to be caused by activation of an intracellular lipase, resulting in mobilization of depot lipids and in lipemia (62). Minute amounts of glucagon are hyperglycemic and lipolytic in ducks, geese, owls, and turkeys and simultaneous injection of insulin does not reduce the response to glucagon (62). Glucagon is so powerfully lipolytic to peripheral tissues in most birds, that the liver removes circulating triglycerides from peripheral fatty tissues and deposits them in the hepatocyte (62). Glucagon may inhibit the hepatic release of lipids. Increased blood glucose levels per se are not responsible for the accumulation of lipid in the avian liver (63,64).

Avian glucagon is potently lipolytic in chickens (65,66) and ducks (67) as chicken adipocytes are more sensitive to glucagon than are rat adipocytes (68). Chicken adipocytes have a significantly higher capacity for the accumulation of cAMP (68). Chicken adipocytes are relatively insensitive to

nonesterified fatty acid (NEFA) inhibition of cAMP synthesis (69).

As for insulin (11), glucagon binding sites are less numerous in chicken versus rat liver (12). In the case of glucagon this has been used to partly explain why the bird has such high circulating concentrations of the hormone, though why this should follow is questioned for it should also apply for insulin. In chicken hepatocytes, the binding of glucagon to every receptor molecule is capable of activating adenylate cyclase (52). Glucagon action is dependent upon its concentration, exerting maximal effect with only 10% of receptors filled (52).

Other hormones such as VIP, secretin, and gut glucagon have no detectable effect on cAMP synthesis in chicken adipocytes (68). Avian growth hormone (GH), at physiologic levels, plays an important role in lipolysis, being extremely lipolytic in chicken adipose tissue (70). There is no stimulatory effect of chicken intestinal glucagon (GLI) on lipolysis in birds (66,71).

Both glucagon and APP, as will be seen later, are glycogenolytic, the latter by an unknown mechanism. Only glucagon-induced hepatic glycogenolysis raises blood glucose levels. Glucose from APP induced glycogen hydrolysis is thought to be shunted to other pathways most likely to fatty acid synthesis (13). Glucagon favours increased gluconeogenesis in the liver of birds by increasing

phosphoenolpyruvate carboxykinase levels and/or activity (12).

The lipolytic enzyme that appears to be directly affected by hormonal action is triglyceride lipase or hormone-sensitive lipase. While glucagon is the main lipolytic hormone, other lesser lipolytic hormones include epinephrine, norepinephrine, secretin, adrenocorticotropin (ACTH), thyroid-stimulating hormone (TSH), and luteinizing hormone (LH). The actions of all these hormones are mediated by cAMP (12,72,73).

Insulin increases fatty acid synthetase activity in cultured avian liver explants while glucagon does not. The increase in synthetase activity is accounted for by an increase in its rate of synthesis (74). Hepatic fatty acid synthetase activity may be prematurely induced in 20-day chick embryos by glucagon indirectly through the elevation of serum insulin levels (75). An elevated plasma insulin/glucagon ratio along with a decreased hepatic cyclic AMP concentration may account for the glycogen and triglyceride storage syndrome in the hypothyroid chick (76). Indeed, since insulin is definitely needed for normal carbohydrate metabolism and glucagon plays a major role in carbohydrate-lipid interactions, many experts feel the circulating glucagon to insulin molar ratio to be <u>the</u> critical controlling factor for normal metabolism in the healthy bird (77).

C. Avian pancreatic polypeptide (APP)

Plasma APP levels range from 8-12 ng/ml in recently fed birds, decrease to 2-4 ng/ml in fasted birds (24hr), and rapidly increase (1-3 min) to 20-30 ng/ml after refeeding has commenced (23). On the contrary in humans, progressive fasting gradually elevates pancreatic polypeptide (PP) levels (78).

Much more is known about PP release than about its biological functions. PP release is regulated by both neurogenic (mainly vagal) pathways and by humoral substances. Upper gut distension leads to an immediate release of PP, which is then followed quickly by a second phase of PP secretion over a period of several hours. Presumably the presence of chyme in the first part of the ileum causes the release of an agent (CCK) to the blood which promotes APP release from the pancreas. Pentagastrin is a powerful stimulant *in vivo* to APP release from the chicken pancreas (23). *In vitro* the most effective nutrient stimulus to APP release is a mixture of amino acids in relative proportion to that found in commercial chicken feed (23). Digesta must pass at least to the level of the ileum for maximal APP expression in response to fasting-refeeding (79).

The physiological significance of this polypeptide in vertebrates remains obscure (80). APP has a distinct receptor (81). The avian liver possesses specific APP-

binding sites of low affinity which may be functionally insignificant (51). The effects of APP are mediated by tissues other than the liver (52). Target organs for APP include pancreas, duodenum, ileum, spleen, and bone marrow, and probably liver and proventriculus. The pancreas, gut, and spleen may be the main target organs for APP in the chicken (82). In vivo injection of APP and bovine PP (BPP) causes among others hepatic glycogenolysis (APP), increased gastric secretion which is reversed with high PP concentrations (APP and BPP), decreased exocrine pancreatic secretion (BPP), increased amino acid incorporation into gastric structural protein (APP), decreased gastrointestinal motility (APP), reduced choleductal tone (BPP), and hypoglycerolemia (APP) (83).

APP is antilipolytic in isolated avian adipocytes and this in vitro effect may partly explain the in vivo hypoglycerolemia caused by exogenous APP (81,13). APP is "insulin-like" in its anti-lipolytic nature. BPP is not as effective as APP in inhibiting glucagon-stimulated lipolysis in the chicken adipocyte. APP may, in fact, react with the glucagon receptor at the adipocyte (48). Other in vivo results have been difficult to confirm in vitro (83).

In non-fasted birds, APP appears to be an anabolic hormone as it encourages a net synthesis of lipid in the liver and its transfer to depot sites with plasma triglycerides rising as a result (23). APP injection into fasted chickens reduces

plasma free fatty acid and glycerol levels (23) and these actions support the *in vitro* observation that APP is anti-lipolytic at the isolated adipocyte (81). APP inhibits adenyl cyclase within avian adipocytes (23). These observations would suggest that a higher circulating APP level might be found in the FL.

APP has been postulated to play a role in the control of hunger-satiety (84,85,83). For instance, obesity can be reversed in ob/ob mice by injection of pancreatic polypeptide. These mice appear to have functioning satiety centres but might lack a circulating satiety factor which could be pancreatic polypeptide (86).

Pancreatic Hormone and Metabolite Interrelationships and Regulation

Intrapancreatic regulation of endocrine secretion occurs in *Aves* (87). Intravenous insulin injection (50 ug/kg b.w.) increases plasma FFA, glucagon and uric acid levels. Glucagon injection (50 ug/kg b.w.) increases plasma insulin and FFA levels as well as glucose and uric acid while it decreases plasma APP concentration. APP injection (50 ug/kg b.w.) increases gastric secretion and is without an effect on plasma glucose and insulin. APP injection has the immediate effect of decreasing plasma FFA levels while surprisingly also increasing glucagon levels in the plasma. (87). It is thus very difficult to determine the exact role

of each hormone as its effects are so interdependent upon circulating metabolites and the status of other hormones.

It was originally thought that, in mammals, interactions between islet hormones took place via a paracrine or intestinal route (19). A second model proposes that insulin serves in the role of release-inhibiting factor for glucagon. This latter model relies on the assumptions that 1) the B-cells are the principal glucose sensors of the islets, and 2) that insulin, a potent inhibitor of glucagon secretion, is the principal regulator of glucagon secretion in the unstressed state (19).

The fourth pancreatic hormone, somatostatin, figures prominantly as a regulatory hormone. For example, there may be a paracrine negative feedback loop between avian D and B (somatostatin-producing) cells (88). In the duck somatostatin exerts, in the embryo and after hatching, a direct and long lasting inhibition of glucose stimulated insulin secretion (89). Somatostatin also continuously inhibits A and B cell output in the avian pancreas. Glucose suppression of glucagon release is at least partially dependent upon local somatostatin secretion (90). The proposed paracrine relationships in birds, as compared to mammals, are shown in figure 4.

In ducks, somatostatin is a potent antilipolytic hormone particularly on the adipose tissue. Somatostatin stimulates IRG secretion at least in part through this inhibition of

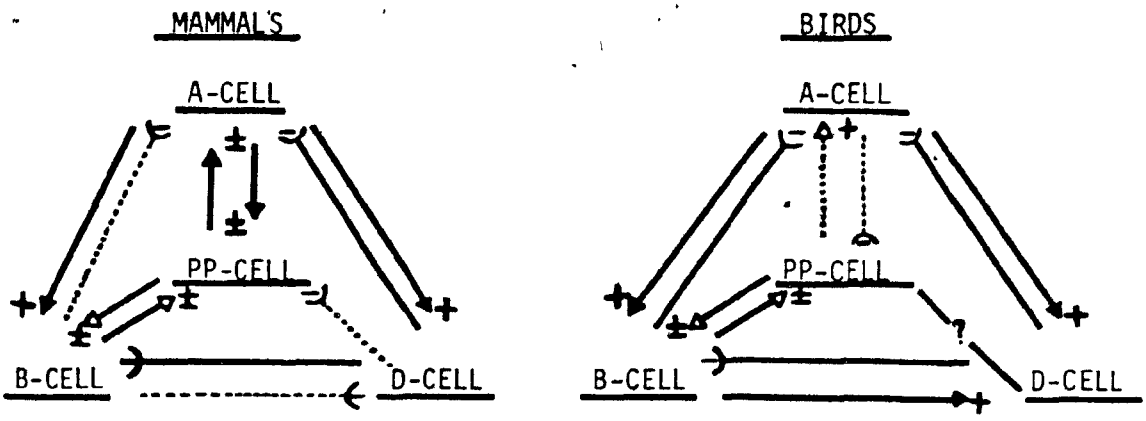


Figure 4 Probable Pancreatic Paracrine Relationship: A Comparison of Mammals with Birds. Legend: solid arrows = strong stimulus, open "cups" = demonstrable inhibition, broken lines = weak effect, ± = no consistent effect. (From Hazelwood, R.L., 1980. The avian gastro-enteric-pancreatic system: structure and function. In "Avian Endocrinology," Epple, A. and M.H. Stetson, (eds.), New York: Academic Press, pg. 231.

lipolysis providing further evidence of the importance of the FFA-glucagon feed-back mechanism in ducks (91).

Glucagon levels are modulated by a direct sensitivity of the avian A cells to glucose (54). For the pancreatic A cell to respond normally to glucose, a normal glucose metabolism within the A cell may be required. Insulin, via its effect on glucose metabolism, appears to modulate glucagon secretion (92), though this remains controversial.

Glucose does not have a direct inhibitory effect on the PP-secreting cells of the chicken pancreas. Volatile fatty acids are not important regulators of insulin, APP or glucagon. Long-chain fatty acids (oleate and linoleate) stimulate insulin release but do not reduce secretion of glucagon or APP by the chicken pancreas (54).

In organ culture, glucose directly stimulates insulin release from splenic lobes of embryos and hatched chicks (93). This observation is in contrast to the transient insulin response obtained in the adult chicken pancreas (41). High glucose concentration does not modify glucagon secretion (93).

In ducks, insulin can lower the plasma FFA level with and without the presence of pancreatic glucagon. This action is glucose dependent (94). Insulin and glucagon may act at the level of reesterification of FFA in liver or adipose tissue (94).

In mammals and birds, alpha receptor activation suppresses glucose-induced insulin release, while beta receptor stimulation increases insulin secretion. In addition, alpha-adrenergic blockade increases basal insulin release in man, while beta-adrenergic blockade decreases it regardless of normoglycemia (56,95). As well, APP secretion from chicken pancreas is regulated by dual alpha- and beta-adrenoreceptor mechanisms (96).

Diabetes in Birds

In the past, the regulation of glucose metabolism in birds appeared to be very different from that described in mammals. "Total" pancreatectomy in birds led to a transient diabetes or to no effect at all. Over the past 20 years or so it has been shown that these so-called "total" pancreatectomies were not, in fact, complete (46,97). According to some there is only a quantitative difference between birds and mammals - a higher plasma glucagon to insulin ratio in birds - and that the basic metabolic regulation is identical in both groups (97).

Two different forms of diabetes have been produced in birds, depending on the completeness of pancreatectomy, i.e. total or subtotal operations. For the purpose of discussion, diabetes will be defined as a pathological or experimental dysfunction of the islets of Langerhans as a whole i.e. a dysfunction of both A and B cells (97).

A. Total pancreatectomy

Results in birds vary according to the species and the authors (97). Most of these "total" pancreatectomies were not complete as subtotal operations (1 to 5% of the pancreas left in place) gave essentially similar results (98,99,100). Parts of the splenic lobe and/or small, unnoticed remnants of pancreas within the duodenal loop may have hypertrophied and led to interpretative difficulties.

Insulin has been extracted from various avian organs (liver, kidney, spleen, etc.) yet insulinogenesis does occur primarily, or possibly exclusively, in the pancreatic B cell. Research results stemming from the use of pancreatropic sulfonylureas in depancreatized birds or use of alloxan and streptozotocin in normal, intact birds have suggested that an extra-pancreatic source of insulin may exist (for reviews see 32,13,101). The goose (and probably the owl) behave more like mammals in their diabetic response to pancreatectomy. It is disquieting that after total pancreatectomy insulin levels can drop by 75% yet persist after 72 hours considering the very short half-life of the chicken hormone (5 min) (102). In a more recent study (103) the existence of extrapancreatic sources of insulin and of glucagon is still proposed. In this latter work APP was the only circulating pancreatic hormone to disappear after pancreatectomy. Perhaps greater differences exist between different species of birds than previously thought, for, in a study by Simon and Dubois (46), the existance of an

extrapancreatic source of insulin is disputed.

Severe hypoglycemia is characteristic after total pancreatectomy in ducks and geese (58,59). Traces of circulating IRI may still be found after pancreatectomy but these authors suggest this may be due to a lack of antibody specificity in the assay and not to an extrapancreatic source of insulin.

After total pancreatectomy in ducks and geese plasma GLI levels drop but do not disappear. That this is gut GLI is suggested using Unger's relatively specific (at least in mammals) antipancreatic glucagon antibody 30K (104,105).

It is the lack of pancreatic glucagon in depancreatized ducks and geese that accounts for the hypoglycemia observed. Glucagon is not replaced by any other endogenous hyperglycemic hormone in spite of the absence of insulin. In normal birds, the plasma glucagon-insulin ratio is one of the critical factors in maintaining a normal plasma glucose level (97).

When pancreatectomized ducks survive long enough (15 to 30 days), their adipose tissue disappears completely, showing that changes in lipid metabolism have occured (97). These changes may have been partly due to the lack of exocrine secretions causing malabsorption resulting in inadequate calories and mobilization of fat.

Hyperaminoacidemia occurs in the chick and goose shortly after pancreatectomy and may be ascribed to the lack of pancreatic hormones for, a glucagon-arginine negative feedback mechanism has been demonstrated in the duck (106) and it is established that insulin inhibits the peripheral release of amino acids and enhances tissue uptake of amino acids (107).

B. Subtotal pancreatectomy

Subtotal pancreatectomy always induces hyperglycemia and diabetes. The diabetes is transient in the duck (98), the chicken (106) and the pigeon (97) while of a permanent nature in the goose (99,58).

i. Transient diabetes- The transient diabetes is characterized by hyperglycemia and impaired glucose tolerance in ducks (98,104) lasting 6 to 12 days after surgery. Thereafter glycemia and glucose tolerance return to normal.

In transiently diabetic ducks the effects of glucose, free fatty acids, and amino acids on insulin and glucagon secretions have been studied (104). No effect of glucose on either insulin or glucagon could be observed. Insulin, in physiological amounts, is required before and after a glucose tolerance test (104). Thus a double action of insulin on the alpha$_2$ cell was postulated: a long term action on, for example, glucose metabolism or glucose

receptors of the alpha cell as well as a short term action via the insulin-glucagon feed-back mechanism. In geese made diabetic by subtotal pancreatectomy there is a progressive loss of sensitivity of the A cell to insulin (109).

While the FFA-glucagon interplay acts during diabetes there is no FFA-insulin feed-back in normal or diabetic ducks (110,111). A normal arginine-glucagon feed-back exists in diabetic ducks while the arginine-insulin interplay is reduced (111).

Circulating glucagon is a prerequisite for a "true" diabetes to appear in all birds. In depancreatized geese glucagon and insulin must be infused in a ratio approximately that found in the plasma of normal fasted animals for the maintenance of normal plasma glucose levels (59).

ii. Permanent diabetes- Permanent diabetes has been observed in granivorous and carnivorous birds although in the latter case further study is required to ascertain that no pancreatic remnant existed after so called total operations (97).

A subtotal operation in geese always induces a permanent diabetes with a survival time rarely exceeding 6 weeks. On the first post operative day, symptoms include hyperglycemia and glucose intolerance concommitant with decreased insulin and glucagon levels. After 3-5 days, glucagonemia progressively rises to reach high levels (99,112). Plasma amino acids increase and do not decrease in response to a

glucose load despite the hyperglucagonemia (97). Lipids are markedly mobilized (112). The glucose-insulin and glucose-glucagon feedback mechanisms do not work in subtotally pancreatectomized geese (97).

In summary then, diabetes is inducible in birds as in mammals but some differences are evident: 1) the glucagon/insulin ratio is higher in birds, a fact which may explain why suppression of both hormones results in hypoglycemia and impaired glucose tolerance; 2) differences in lipid metabolism perturbations between birds and mammals following pancreatectomy may be due to the absence or important reduction of the anti-lipolytic effect of insulin (97).

Fat Metabolism and the FL/LL Model

It is apparent that the pancreatic hormones have complicated and interconnected roles. Before continuing a discussion of the FL/LL situation with respect to these hormones the physiological aspects of body fat deposition will be covered in brief.

Fat depots increase in size either by adipocyte multiplication (hyperplasia) or by lipid accumulation within the cells (hypertrophy)(50). Hyperplasia is important during the first few weeks of growth in the broiler chicken and is observed up to 15 weeks of age (113,114,115). Thereafter, hypertrophy accounts for all increases in adipose tissue

mass.

Adipose tissues are composed of many cell types. Adipocytes are derived from perivascular cells (pericytes). Pericytes are specialized to form adipoblasts and then into the fat storing preadipocytes. Preadipocytes enlarge to become adipocytes according to nutritional conditions and endocrine control. It should be mentioned that nutritional conditions do not have persistent effects on adiposity in birds contrary to the situation in rats where overnutrition or undernutrition during the hyperplastic growth stage may increase or decrease, respectively, the number of fat cells in the adult (50).

Different regulatory systems are presumed to be associated with each adipose tissue. For instance, subcutaneous fat develops earlier than abdominal fat in mammals (50) and in birds (116). As well, different adipocytes have varying sensitivities to the hormones controlling lipolysis and fat storage (Simon, 1984 pers. comm.).

Lipolysis and lipogenesis occur at the same time. Net lipolysis tends to result in a reduction of adipose tissue while net lipogenesis results in an increase in adipose tissue mass. The control of lipolysis has already been discussed (50).

Adipose tissue growth involves three main stages: 1) hepatic lipogenesis, 2) transport of lipids as lipoproteins (very

low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) etc.) and 3) the removal of plasma triglycerides by adipocytes. Insulin has been thought to be the major hormone associated with the control of lipogenesis (50).

Triglycerides are removed from the plasma via lipoprotein lipase activity (LPL). Insulin stimulates LPL activity in both chicken and turkey adipose tissue in vitro (117) and in mammals (118).

In chickens, plasma (VLDL+LDL) triglyceride concentrations have been shown to be correlated with body fat (119). VLDL and HDL concentrations were higher in the FL birds whether fed or starved (50). This fact was in keeping with the increased hepatic fatty acid synthesis in the fat line (120). Accumulation of VLDL in plasma may have been due to increased splanchnic secretion or reduced peripheral removal with the former mechanism normally predominating in the chicken (50).

These fat and lean birds have been compared to the Zucker rat (50) with respect to fat metabolism. The main similarities between these two models were higher insulin secretion, high plasma triglycerides, high VLDL, high plasma phospholipids and lower plasma free amino acid concentrations (121) as well as enhanced hepatic lipogenesis, and less efficient protein utilization.

Differences between the models included glycemia, glucose disposal rate, body temperature, maintenance energy requirement, and plasma cholesterol (56). Even though rodent obesity is monogenic, the events associated with it (figure 5) have been used to help understand the polygenic fattening of avian species (2).

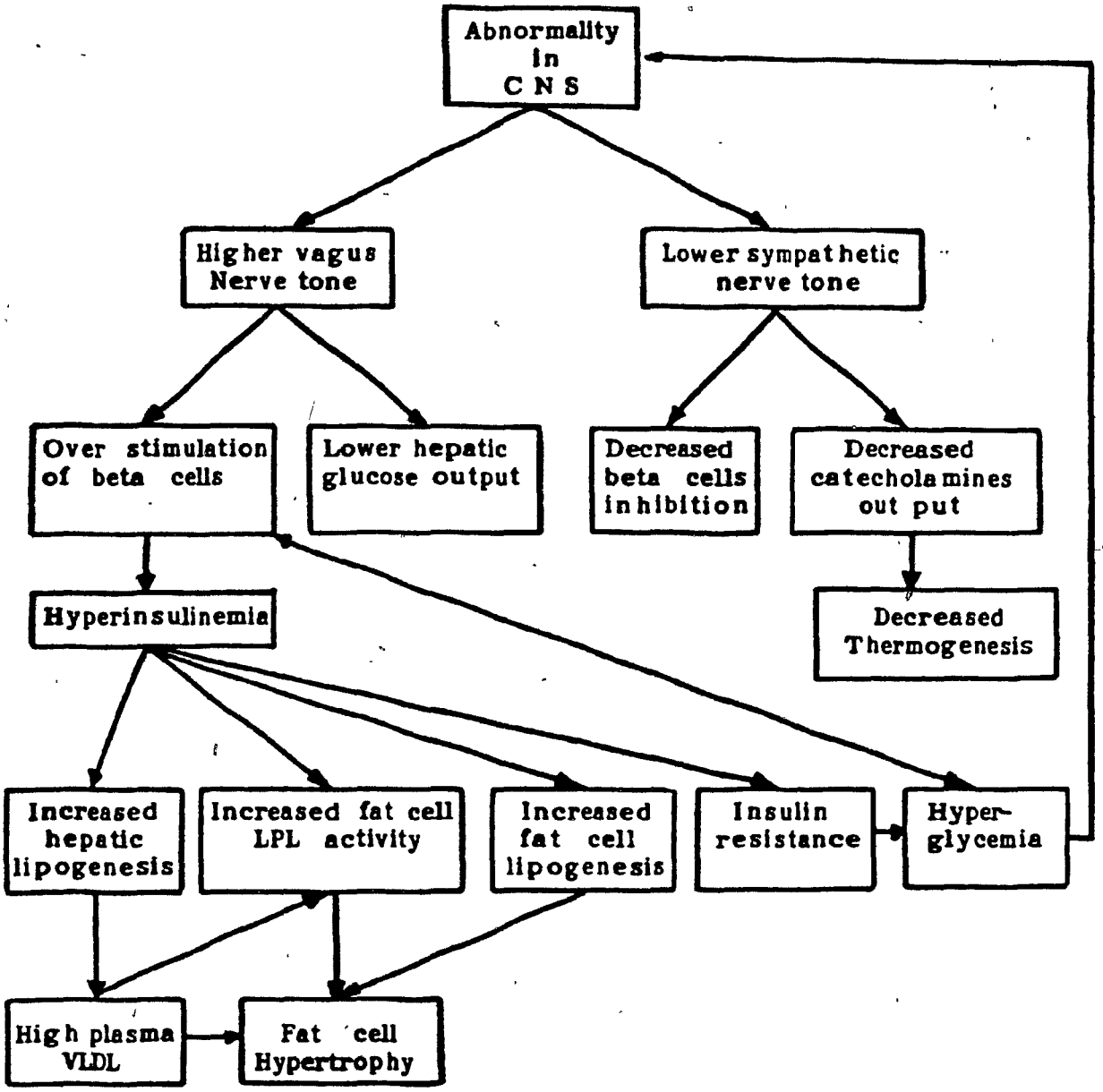


Figure 5 Possible succession of events leading to obesity in rodents. (From LeClercq, B., 1985. The genetic and physiological basis of fattness in poultry. 7th European Symposium on Poultry Meat Quality, Denmark (in press).)

Hypotheses

Insulin has been shown to be involved in the fattening of the FL yet insulin is thought to play a secondary role to glucagon in birds. As glucagon appears to be the major pancreatic hormone in birds it was hypothesized that this hormone would be of greater importance in the control of fat deposition via its lipolytic/antilipogenic action. A lesser rate of lipolysis might be more important than the increased rate of lipogenesis in causing the increases in adipose tissue mass in the FL. Therefore it was hypothesized that the LL would have, in general, higher circulating levels of pancreatic glucagon compared to the FL. In addition, a lower circulating glucagon to insulin molar ratio (GTIR) was proposed for the FL over the LL that would result in greater net lipogenesis and less lipolysis in the FL.

Little is known about APP though its anti-lipolytic and lipogenic actions would lead one to hypothesize that increased levels of APP might be found in the FL.

Novelty of Hypotheses

The novelty of these hypotheses was that firstly, there was no precedent in mammals for it. Kalkoff (122) stated that, in the obese human, hyperinsulinemia appeared to be a principal metabolic observation whereas plasma glucagon disturbances were inconsistent. The ob/ob mouse, the db/db mouse, and the Zucker rat, do have abnormal glucagon secretion but this may be due to their diabetic state rather than to their obesity. Secondly, no glucagon research has been done on these FL/LL chickens. Lastly, there has been no published work on APP differences between these two lines.

EXPERIMENTAL DESIGNS AND SAMPLING PROCEDURES

Experiment 1

Rationale- This experiment was undertaken to observe whether the FL and LL fed and/or fasted chickens differ in plasma levels of glucose, free fatty acids, glucagon, insulin, and avian pancreatic polypeptide. As dietary manipulations alter circulating levels of the pancreatic hormones (123) an added treatment of fat versus non-fat supplemented diets was implemented to try and bring out any line differences.

Therefore, at one day of age, FL and LL chicks of mixed sex, from the F6 generation of French broiler breeders reared at the Macdonald College Poultry Unit, were placed in an electrically heated Petersime battery brooder with raised wire floors. Ninety-six birds from each line were randomly allotted to 12 pens to arrive at 8 birds per pen per line.

The birds were raised to 4 weeks of age (average body weight 450-500 g) on semi-purified chick starter diets containing either 9% fat (high fat diet) or 2% fat (low fat diet). The compositions of the experimental diets are given in table 2. Feed and water were provided ad libitum. Feed consumption was measured for each pen. The brooder temperature was maintained at 30°C for the first week and was decreased by 2°C/week to four weeks of age.

At 4 weeks of age the birds were weighed and blood samples

were taken. They were killed by cervical dislocation and their pancreata removed. On the first day samples were taken from fed birds (4 birds per pen). The feed was then removed and on the following day, after a 16 hour fast, samples were taken from the remaining birds. Care was taken on the first day to remove a representative sample with respect to sex and body weight from each pen. There were thus 16 treatments: 2 lines x 2 diets x 2 states (i.e. fed or fasted) x 2 sexes.

Sampling procedure:

Ten c.c. of blood were removed by cardiac puncture into heparinized syringes using "Hepalean" (Organon; 10,000 USP units/ml) as anticoagulant. The blood was held in a bath of crushed ice prior to centrifugation. Within 45 minutes of removal the blood was centrifuged (700 x g) under refrigerated conditions to separate the plasma.

Aliquot samples of plasma were frozen for future analyses for glucose, glucagon, insulin, avian pancreatic polypeptide, and free fatty acids. The aliquots for plasma glucagon analyses contained the proteinase inhibitor, Aprotinin ("Trasylol"R) (Miles Laboratories, Toronto cat. no. 817113) at 10% v/v. Pancreata were rinsed with saline, weighed, and placed in individual screw top vials and frozen in liquid CO_2. The carcasses were laid out in a cold room overnight and their abdominal fat pads were removed and weighed the following day.

Table 2: Composition of semi-purified diets, Experiment 1

	Non-fat supplemented	Fat supplemented
Isolated soybean protein (Promosoy 100)	33.92	33.92
D-glucose, anhydrous	27.79	19.36
Corn starch	28.05	19.64
Refined corn oil (Mazola)	2.00	2.00
Animal-vegetable fat blend[1]	---	7.00
DL-methionine	0.31	0.31
L-glycine	0.25	0.25
Vitamin premix[2]	0.50	0.50
Mineral premix[2]	5.65	5.65
Ground cellulose (Alphacel)	1.53	11.35
% determined crude protein	22.44	22.37
% determined ether extract	1.96	8.33

[1]The animal-vegetable fat blend had a gross energy of 9,213 kcal/kg, an iodine number of 69 and a melting point of 24C. Its fatty acid composition by analysis was: myristic 1.3%, palmitic 22.6%, palmitoleic 4.3%, stearic 11.5%, oleic 47.6%, linoleic 11.2% and linolenic 0.8%.

[2]As per Scott, M.L., M.C. Nesheim and R.J. Young, 1982. Nutrition of the Chicken. 3rd edition, M.L. Scott and Associates, Publishers, Ithaca, N.Y.

Experiment 2

Rationale- Cold stress and hypothermia are both associated with severe carbohydrate intolerance. In cold stress, metabolism increases as a result of activation of the sympathetic nervous system and by shivering (124). Plasma glucagon levels are known to increase under stress. With this in mind, experiment 2 was designed to again try and bring out any between line differences in the pancreatic hormones.

A total of 80 birds was used. Forty day-old chicks per line were assigned completely at random to 4 pens of the battery brooder. Rearing conditions were similar to those for experiment 1 except that a single commercial diet (Co-Op Federeé broiler starter mash calculated to contain 3125 kcal of metabolizable energy/kg and 22% crude protein) was fed. Feed and water were provided ad libitum. Temperature was as for Experiment 1. The chicks were reared to 5 weeks of age (average body weight 550-650 g).

On the sampling day, following a 16 hour fast in 2 of the 4 pens per line, half of the birds from each pen were removed to a refrigerated room (5°C) for a 2-3 hour period prior to sample collection. There were 16 treatments: 2 lines x 2 temperatures x 2 states (i.e. fed or fasted) x 2 sexes.

Blood sampling, pancreas and abdominal fat removal were as previously described in Experiment 1.

Experiment 3

Rationale-The following experiment was designed in an attempt to bring out any between-line differences in APP. As reported by Dr. J.R. Kimmel (79,82), the maximal response in APP release from the avian pancreas is obtained upon refeeding following a 16 hour fast. Therefore, in this experiment, it was the intention to determine any line differences in plasma APP following normal feeding, fasting, and fasting then refeeding.

A total of 60 birds was distributed into 12 pens. Thirty birds per line were assigned completely at random to 6 of the 12 pens. As in the previous trial, the chicks were raised in a battery brooder from one day to five weeks of age on a commercial starter diet. Feed and water were provided ad libitum. Temperature was controlled as in experiment 1. At 5 weeks of age birds in 2 of the 6 pens were fasted (16 hours), 2 were fed, and 2 were fasted for 16 hours then refed (45 minutes) prior to sampling. There were thus 12 treatments: 2 lines x 3 states (fed, fasted, refed) x 2 sexes.

Blood sampling, pancreas and abdominal fat removal were as previously described (Experiment 1).

LABORATORY ANALYSES

Plasma glucose concentrations were measured by the glucose oxidase technique using the Beckman II Glucose Analyzer (Beckman Instruments, Fullerton, California). Plasma free fatty acids were estimated by a radiochemical microtechnique using ^{63}Ni(125). Plasma levels of avian pancreatic polypeptide were measured by Dr. Kimmel (Veteran's Administration Medical Centre, Kansas City, MO.) according to his own method (126). For measurement of pancreatic levels of any of the various hormones, the pancreatic cells were first disrupted (Sonifier Cell Disrupter 350, Branson Sonic Power Co.) and the hormones extracted overnight at 4°C in an acid-ethanol mixture (27). The extracts were then serially diluted and assayed for immunoreactive insulin and/or glucagon.

Insulin Radioimmunoassay

A heterologous radioimmunoassay was conducted for chicken insulin following, in part, the method used by Marliss (127). Chicken insulin standard was received as a gift from the National Institute of Child Health and Human Development (who originally obtained it from Litron Laboratories, Rochester, N.Y.). This was reconstituted in 1 ml of assay buffer (see below). No contamination with glucagon, APP, or somatostatin was claimed by Litron. ^{125}I-labelled porcine insulin was purchased from Novo Research Institute, Denmark.

A guinea pig anti-beef insulin antibody (a 1:100 dilution) was obtained from the University of Vermont's General Clinical Research Centre, Burlington, Vermont, where it had been obtained previously from Dr. P.H. Wright.

Reagents- For the dilution of anti-beef insulin antisera, ^{125}I-labelled porcine insulin, and chicken insulin standard, a glycine assay buffer (0.4M Glycine, BDH Chemicals, Montreal; pH 8.8, 0.2M) containing normal lamb serum (1% v/v Gibco Laboratories, Grand Island, New York) and bovine albumin (0.25% w/v; fraction V; Sigma Chemical Company, St. Louis, Missouri) was used.

Insulin antibody- Wright antisera received as a 1:100 dilution, was diluted to 1:1000 with assay buffer and stored at -70°C (stock solution). To obtain the working solution, 100 microliters of the stock solution was diluted to.16 ml with assay buffer to arrive at a final dilution of antibody in the assay mixture of 1:800,000.

Radiolabelled insulin- ^{125}I-labelled insulin (0.82 micrograms insulin content; 25.7 microCi radioactivity per vial; 31.3 microCi/microgram of insulin specific radioactivity; 10 mg human albumin content) was obtained (3 or 4 batches) from Novo and reconstituted in 5 ml. of assay buffer, divided into 120 microlitre aliquots (stock solution) and stored at -20°C. Before use, 100 microlitres of stock solution were diluted to 60 ml with assay buffer.

Insulin standard-The gift of chicken insulin standard (NICHD)(lot no. 109 Litron Laboratories) was received in a lyophilized form and was reconstituted in 1.0 ml of assay buffer to arrive at a stock solution of 100 micrograms per ml. The stock solution was stored as 200 microlitre aliquots at -20 °C in Beckman microfuge tubes. For purposes of the assay 100 microlitres of stock solution were diluted to 10 ml with assay buffer to give a working standard solution of 10 ng/ml. Stock solutions were diluted immediately before use in each assay to avoid loss of activity at the lower concentrations.

Assay Procedure:

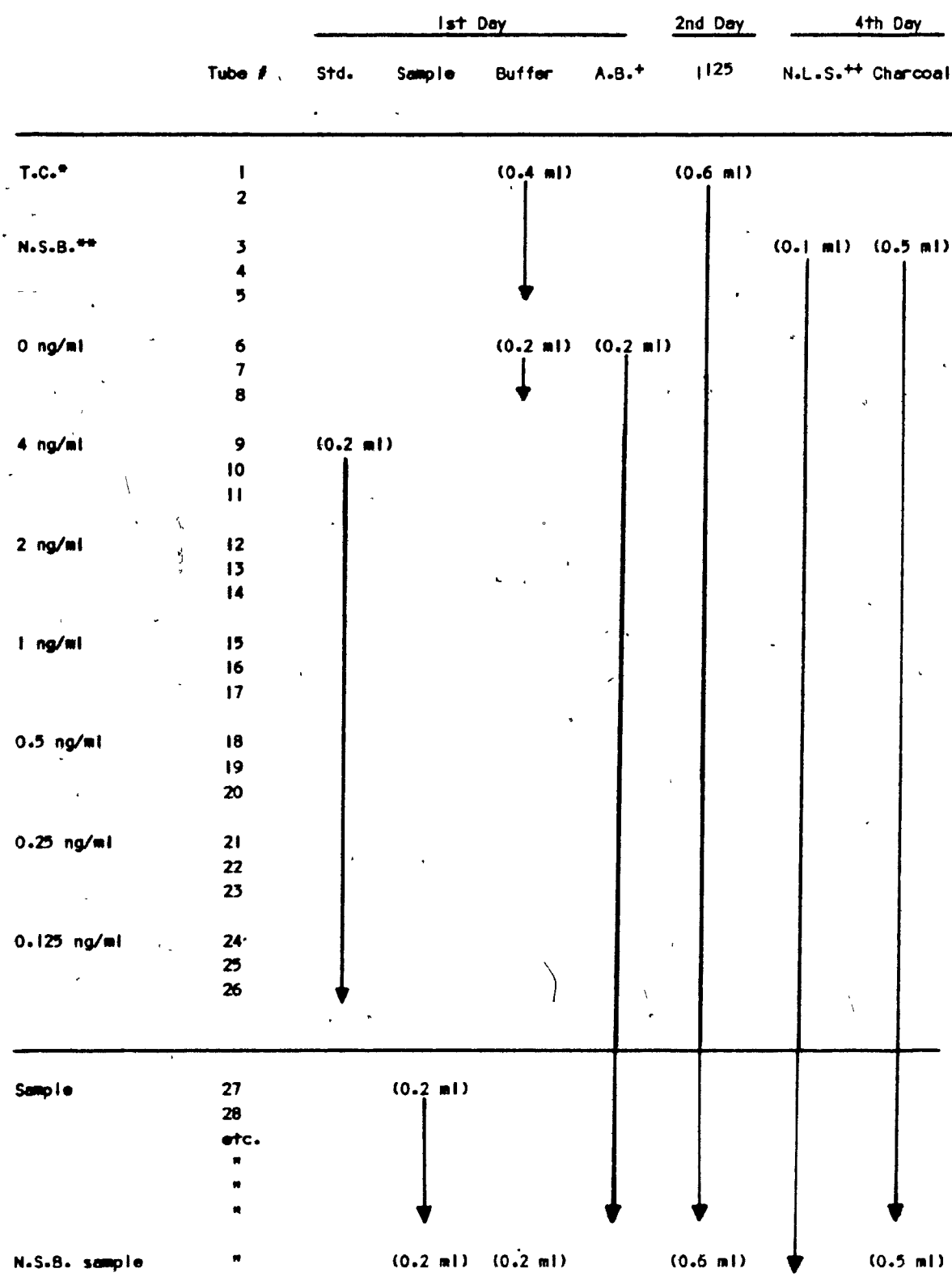
1st day:preparation of antibody and standards which were kept at 4°C. All incubations were carried out in 12X75 mm glass culture tubes to which buffer, samples, or standards were first added according to table 3 . Antibody was added secondly to all tubes except to tubes for total counts and non-specific binding. Tube contents were then mixed (SMI model 2600 multi-tube vortexer) and incubated for 24 hours at 4°C.

2nd day: ^{125}I-labelled insulin was prepared by appropriate dilution and added to all tubes. The tube contents were then mixed and incubated 48 hours at 4°C.

4th day: A suspension of dextran-coated charcoal was prepared. Charcoal 3% w/v (Norit A, Fisher Scientific Co.,

U.S.A. cat. no. C-176) plus 0.25% w/v Dextran T-70 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) were prepared in 100 ml of 0.4M Glycine plus 100 ml distilled water and allowed to stir, using a magnetic stirrer, at least 1 hour before addition. Before charcoaling, normal lamb serum was thawed and added to tubes 3-36 to equalize the protein concentration of the standards to that of the sample tubes. The tube contents were then mixed. Then the charcoal-dextran mixture was added to all tubes except those for total counts. Tubes were gently mixed and incubated for 30 minutes at 4°C then centrifuged for 15 minutes at 3000 rpm (2000 x g) at 4°C. The supernatants were then aspirated and the charcoal precipitates which represent unbound ^{125}I-insulin were counted in the gamma counter (LKB Wallac 1271 Riagamma Automatic Gamma Counter). The percent bound for each sample was calculated by subtracting from total counts those for the sample precipitate and then dividing by the total counts. Unknown insulin concentrations were read off the standard curve.

Table 3. Insulin radioimmunoassay procedure



* Total counts ** Non specific binding + Antibody ++Normal lamb serum

Results:

A typical standard curve is shown (figure 6). Peter Wright's anti-beef insulin antibody would appear to give a favourable cross reactivity with chicken insulin. At 0 ng/ml of chicken insulin the percent of bound labelled insulin was about 78%. At 4 ng/ml of chicken insulin standard, the percentage of bound labelled hormone was approximately 20%. Normal physiological levels for chicken insulin range from 0.15 ng/ml to 1.5 ng/ml and thus the critical portion of this dose-response curve would be of practical use for avian plasma samples without any dilutions being necessary. Dilutions of chicken pancreatic extract were measured for their insulin content using this method and were found to fall very well upon the critical portion of this standard curve.

A pooled sample of chicken plasma was used to test for inter-assay reproducibility. The standard had an insulin concentration of 0.81 ± 0.02 ng/ml. The intra-assay variation was less than 5%.

Detection of concentrations of chicken insulin as low as 0.03 ng/ml and up to 4 ng/ml is possible using this assay. The advantage of this method is that the antibody, standard and tracer are all available and thus no labourious preparation is necessary. There is also no need to purchase a second antibody as for a double-antibody technique.

An anti-chicken antibody obtained from Dr. J.P. McMurtry (Beltsville Agricultural Research Centre, Beltsville, Maryland 20705) (at a 1:15,000 dilution) was also tested against the same ^{125}I-anti-porcine insulin tracer. The dose response curve obtained was poor ranging from 37% bound to 5% bound (not shown). Whether the antibody preparation was poor or did not react well with the tracer is unknown. It is, though, inferior to the Wright antibody in terms of the quantity required to conduct an assay. Very high concentrations of this chicken antibody would be required to improve its performance in the assay described herein.

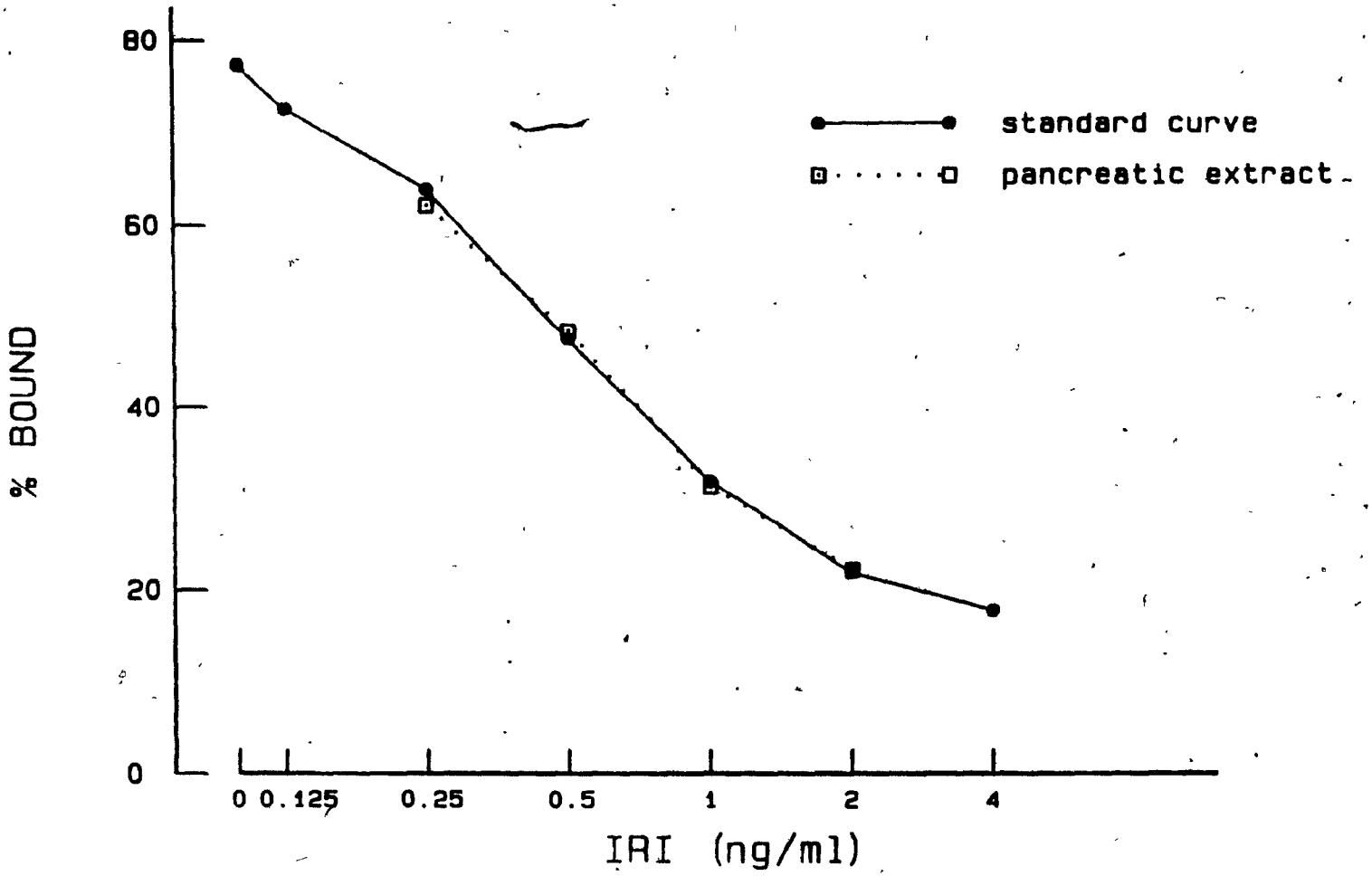


Figure 6 Insulin standard curve obtained using a chicken insulin standard, a guinea pig antibeef insulin antibody and a ^{125}I-labelled porcine insulin tracer. Superimposed on this graph is another curve obtained by dilution of a chicken pancreatic extract to show the lack of a dilution effect and the ability of this assay system to measure avian insulin.

Glucagon Radioimmunoassay

A heterologous radioimmunoassay was conducted for pancreatic glucagon following, in part, the method used by Nakhooda et al. (128). Crystallized, highly purified porcine glucagon (100 micrograms) was obtained from Novo Research Institute, Denmark (lot no. G421306) containing 0.002% insulin and 15.72% nitrogen. ^{125}I-labelled porcine glucagon was also purchased from Novo. The antibody used was 30K from the University of Texas Health Science Center at Dallas, U.S.A.

Reagents- For the dilution of antibody, ^{125}I-labelled porcine glucagon, and pancreatic glucagon standard a glycine buffer (BDH Chemicals, Montreal; pH 8.8, 0.2M) containing human lamb serum (1% v/v)(Gibco Laboratories, Grand Island, N.Y.) was used.

Glucagon antibody- Twenty-five microlitres of rabbit beef-pork glucagon antiserum (30K) were received in a lyophilized form. The 30K is claimed to have a high specificity for pancreatic glucagon. It was reconstituted in 5ml of assay buffer to obtain a 1:200 stock solution which was then divided into aliquots and stored at -20°C. The stock solution was diluted to 1:100,000 for use in the assay.

Radiolabelled glucagon- ^{125}I-porcine glucagon was obtained from Novo. The 0.075 micrograms of lyophilized radioactive glucagon was reconstituted in 1.0 ml of assay buffer, aliquoted as stock solution and stored at -20°C. For use in

the assay, 100 microlitres of stock solution plus 20 ml of Trasylol was diluted to 120 ml with assay buffer. This dilution was such that 18 pg of labelled glucagon would be present in 0.6 ml taken for the assay.

Glucagon standard- Master stock (100 ug/ml), stock (1 ug/ml), and working solutions (2000 pg/ml) of highly purified porcine glucagon were stored at -70°C. For the assay, dilutions of 2000, 1000, 500, 300, 200, 100, 50, and 20 pg/ml were used.

Assay Procedure:

First day:

Added to 12 x 75 mm glass culture tubes:

1) 0.6 ml ^{125}I-glucagon containing Trasylol
2) 0.2 ml standard or samples (assay standards in triplicate; samples in duplicate)
3) 0.4 ml antiserum

   1.2 ml total volume.
4) Non-specific binding: Tubes were set up containing assay diluent rather than antiserum for the standard curve and for each group of samples to allow for correction for differences in the non-specific binding.
5) Counting standards: Tubes representing total counts were set up with assay buffer in place of antiserum, plus sample or standard.
6) Tube contents were then mixed and incubated at 4°C for 4 days.

Fourth day:

7) A suspension of dextran-coated charcoal (0.5% charcoal w/v, 0.25% dextran w/v) was prepared by mixing 1% Norit A charcoal and 0.5% Dextran-70 in 0.2M glycine (pH 8.8). The mixture was stirred at least 45 minutes prior to use.

8) Before addition of charcoal mixture, 0.2 ml of normal lamb serum (1% v/v Gibco Laboratories, Grand Island, N.Y.) was added to samples containing no plasma for the purpose of standardization.

9) 0.5 ml of the dextran-coated charcoal suspension was added to each tube except those for total counts. They were mixed and left to incubate for 45 minutes at 4 °C.

10) Tubes were then centrifuged for 15 minutes at 4°C at 3000 rpm (2000 x g).

11). The supernatant was aspirated by suction. Tubes containing the charcoal pellets and those for total counts were then counted in a gamma scintillation counter (LKB Wallac 1271 Riagamma Automatic Gamma Counter). Calculations were done as for IRI. Unknown glucagon concentrations were read off the standard curve.

Results:

A typical standard curve is shown (figure 7). The 30K antibody used thus appears to have given favourable cross-

reactivity with the mammalian standard employed. At a concentration of 0 pg/ml porcine glucagon, the percentage bound was approximately 65%. At 2000 pg/ml of porcine glucagon the percentage of bound radiolabelled glucagon had dropped to 10%

The most sensitive portion of the curve i.e. the portion of the curve with the greatest slope, was found to lie between 100 pg/ml and 500 pg/ml. Chicken plasma samples were diluted to the point that they could be read from this critical region. Dilutions of 1:4 and 1:8 for chicken plasma were usually found satisfactory. A dilution effect was not evident in most cases.

This radioimmunoassay system was tested for its ability to measure avian pancreatic glucagon. Acid-ethanol extracts of avian pancreata and plasma samples were serially diluted and measured by RIA for glucagon. It was found that the extracts and plasma produced a response pattern very similar to that of the mammalian standard. The 2 curves could almost be superimposed.

On a pooled plasma control, this method gave an inter-assay reproducibility error of less than 12% with an intra-assay error of less than 5%.

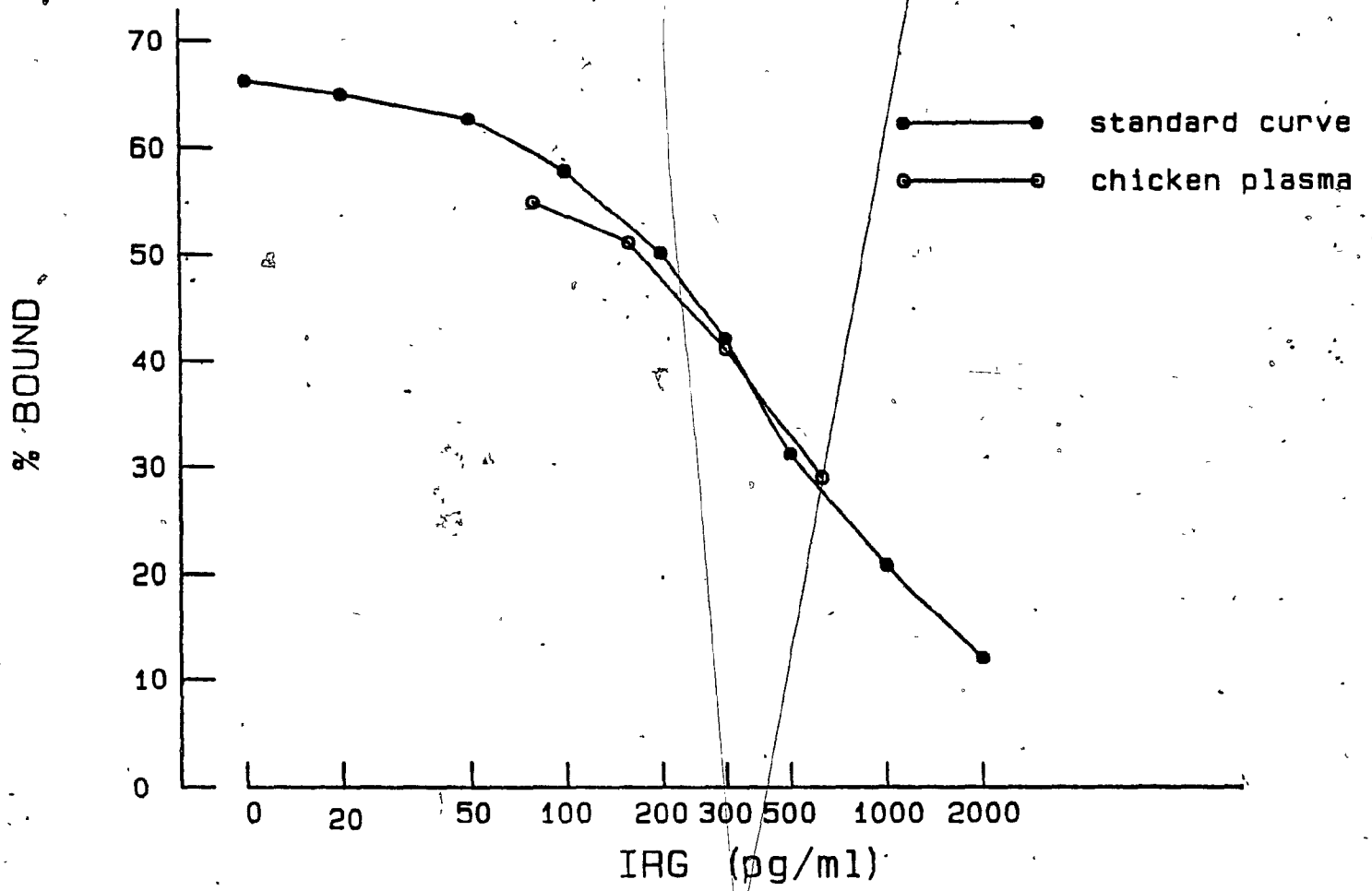


Figure 7 Glucagon standard curve obtained using a porcine glucagon standard a rabbit beef-pork glucagon antibody and a ^{125}I-labelled porcine glucagon tracer. Superimposed on this graph is another curve obtained by dilution of a representative chicken plasma sample to show the lack of a dilution effect and the ability of this assay system to measure avian glucagon.

CALCULATIONS FOR DATA ANALYSIS AND STATISTICAL METHODS OF DATA ANALYSIS

The glucagon to insulin molar ratio (GTIR) was calculated as follows:

MW of glucagon = 3500 daltons
and MW of insulin = 6500 daltons

therefore molar ratio =

$$\frac{\text{concentration glucagon (ng/ml)}}{\text{concentration insulin (ng/ml)}} \times \frac{6500}{3500}$$

The percent abdominal fat (PAF) was expressed on a live weight basis.

The results were analyzed using the GLM procedure of the SAS package (129). The 4 model equations used to analyze the data are given on the following pages. The formulae were used to provide least-square means for the various treatments (main effects) and the various interactions (fixed effects). The experiment 2 data were analyzed with and without the cold room data to separate out effects that may have been due to the cold only. For ease of reporting, many of the interactions judged to be insignificant at the 0.05 probability level were not included in the tables of results.

The model equations used to calculate the correlations included all main effects but only those interactions that were found to be significant. The partial correlation equations are not given. The partial correlations given are from the error sums of squares and cross products matrix in the SAS package.

Model equation for experiment 1:

$$Y_{ijklm} = u + L_i + D_j + St_k + S_l + LD_{ij} + LS_{il} + DSt_{jk} + DS_{jl} + StS_{kl} + LDSt_{ijk} + PAF \cdot x_{ijklm} + e_{ijklm}$$

where:

Y_{ijklm} = the $ijklm^{th}$ observation of blood parameters (e.g. glucose, insulin etc.)

u = the population mean

L_i = effect of line ($i=1,2$)

D_j = effect of diet ($j=1,2$)

St_k = effect of state (fasted, fed) ($k=1,2$)

S_l = effect of sex ($l=1,2$)

LD_{ij} = interaction of the i^{th} line effect and the j^{th} diet effect

LSt_{ik} = interaction of the i^{th} line effect and the k^{th} state effect

LS_{il} = interaction of the i^{th} line effect and the l^{th} sex effect

DSt_{jk} = interaction of the j^{th} diet effect and the k^{th} state effect

DS_{jl} = interaction of the j^{th} diet effect and the l^{th} sex effect

StS_{kl} = interaction of the k^{th} state effect and the l^{th} sex effect

$LDSt_{ijk}$ = interaction of the i^{th} line effect and the j^{th} diet effect and the k^{th} state effect

PAF = the partial regression on percent abdominal fat

x_{ijklm} = the percent abdominal fat for the $ijklm^{th}$ individual

e_{ijklm} = error term.

Model equation for experiment 2:

$$Y_{ijklm} = u + L_i + T_j + St_k + S_l + LT_{ij} + LSt_{ik} + LS_{il} + TSt_{jk} + TS_{jl} + StS_{kl} + LTSt_{ijk} + PAF \cdot x_{ijklm} + e_{ijklm}$$

where:

Y_{ijklm} = the $ijklm^{th}$ observation of blood parameters (e.g. glucose, insulin etc.)

u = the population mean

L_i = effect of line ($i=1,2$)

T_j = effect of temp ($j=1,2$)

St_k = effect of state (fasted, fed) ($k=1,2$)

S_l = effect of sex ($l=1,2$)

LT_{ij} = interaction of the i^{th} line effect and the j^{th} temperature effect

LSt_{ik} = interaction of the i^{th} line effect and the k^{th} state effect

LS_{il} = interaction of the i^{th} line effect and the l^{th} sex effect

TSt_{jk} = interaction of the j^{th} temperature effect and the k^{th} state effect

TS_{jl} = interaction of the j^{th} temperature effect and the l^{th} sex effect

StS_{kl} = interaction of the k^{th} state effect and the l^{th} sex effect

$LTSt_{ijk}$ = interaction of the i^{th} line effect and the j^{th} temperature effect and the k^{th} state effect

PAF = the partial regression on percent abdominal fat

x_{ijklm} = the percent abdominal fat for the $ijklm^{th}$ individual

e_{ijklm} = error term.

Model equation for experiment 2 without the cold room data:

$$Y = u + L_i + St_j + S_k + LSt_{ij} + LS_{ik} + StS_{jk} + PAF \cdot x_{ijkl} + e_{ijkl}$$

where:

Y_{ijkl} = the $ijkl^{th}$ observation of blood parameters (e.g. glucose, insulin etc.)

u = the population mean

L_i = effect of line ($i=1,2$)

St_j = effect of state ($j=1,2$)

Sk = effect of sex ($k=1,2$)

LSt_{ij} = interaction of the i^{th} line effect and the j^{th} state effect

LS_{ik} = interaction of the i^{th} line effect and the k^{th} sex effect

StS_{jk} = interaction of the j^{th} state effect and the k^{th} sex effect

PAF = the partial regression on percent abdominal fat

x_{ijkl} = the percent abdominal fat for the $ijkl^{th}$ individual

e_{ijkl} = error term.

Model equation for experiment 3:

$$Y_{ijkl} = u + L_i + St_j + S_k + LSt_{ij} + LS_{ik} + StS_{jk} + LStS_{ijk} + PAF \cdot x_{ijkl} + e_{ijkl}$$

where:

Y_{ijkl} = the $ijkl^{th}$ observation of blood parameters (e.g. glucose, insulin etc.)

u = the population mean

L_i = effect of line ($i=1,2$)

St_j = effect of state (fasted, fed, refed) ($j=1,2,3$)

S_k = effect of sex ($k=1,2$)

LSt_{ij} = interaction of the i^{th} line effect and the j^{th} state effect

LS_{ik} = interaction of the i^{th} line effect and the k^{th} sex effect

StS_{jk} = interaction of the j^{th} state effect and the k^{th} sex effect

$LStS_{ijk}$ = interaction of the i^{th} line effect and the j^{th} state effect and the k^{th} sex effect

PAF = the partial regression on percent abdominal fat

x_{ijkl} = the percent abdominal fat for the $ijkl^{th}$ individual

e_{ijkl} = error term.

RESULTS

Overall percent abdominal fat results:

The percent abdominal fat results are shown for all three experiments (table 4). In all experiments, the percent abdominal fat was significantly higher in the FL compared to the LL (2-3 times greater) and significantly higher in females versus males (1.2-1.3 times greater in females). In Experiment 1, the chicks that consumed the fat supplemented diet had significantly less abdominal fat than the chicks that consumed the non-fat supplemented diet (0.8 times less).

Table 4: The effect of line (expts. 1, 2, & 3), dietary fat supplemention (expt. 1), and sex (expts. 1,2,& 3) on percent abdominal fat (PAF).

	PAF (% live b.w.)		
	EXPT. 1[1] (4 week old)	EXPT. 2[2] (5 week old)	EXPT. 3[3] (5 week old)
LINE			
FL	1.5 ± 0.1^{a}	2.5 ± 0.1^{a}	2.5 ± 0.1^{a}
LL	0.5 ± 0.1^{b}	0.9 ± 0.1^{b}	1.1 ± 0.1^{b}
	(0.0001)	(0.0001)	(0.0001)
DIET			
FS	0.9 ± 0.1^{b}		
NFS	1.1 ± 0.1^{a}		
	(0.0007)		
SEX			
F	1.2 ± 0.1^{a}	1.6 ± 0.1^{a}	1.9 ± 0.1^{a}
M	0.9 ± 0.1^{b}	1.5 ± 0.1^{b}	1.5 ± 0.1^{b}
	(0.0001)	(0.0147)	(0.0130)

[1] mean±sem for 92-96 chicks.
[2] mean±sem for 38-40 chicks.
[3] mean±sem for 28-30 chicks.
Numbers in parentheses indicate the probability of a greater F value.
^{a,b} means for any treatment not having the same superscript are significantly different.
FS= fat supplemented.
NFS= non-fat supplemented.

In the following presentation of results, unless stated otherwise the percent abdominal fat (PAF) was included as a covariate in the model equations for all 3 experiments.

EXPERIMENT 1

The results for plasma glucose, glucagon (IRG), and insulin (IRI) are presented in table 5.

GLUCOSE- Glucose levels varied with line ($p=0.0004$), diet ($p=0.0265$), and state ($p=0.0001$). The FL had lower (228 mg%) and the LL higher (251 mg%) plasma glucose levels. Fat supplemented diets were associated with higher (245 mg%) and non-fat supplemented diets with lower (234 mg%) plasma glucose levels. Fasting plasma glucose levels were lower than fed levels 211 versus 266 mg% respectively. There was no effect of sex on circulating glucose levels.

GLUCAGON- There was a trend for plasma glucagon levels to be affected by line ($p=0.0655$). Fat line plasma glucagons averaged 1.71 ng/ml while the lean line averaged 2.09 ng/ml.

INSULIN- Plasma insulin levels varied with state ($p=0.0001$). There was also a significant line x state interaction ($p=0.0296$). Fasted birds averaged plasma insulins of 0.45 ng/ml while fed birds averaged 0.98 ng/ml. FL and LL fasted birds did not differ significantly in plasma insulin levels (0.50 and 0.41 ng/ml respectively). However, in the fed state, LL birds had significantly higher (1.10 ng/ml) plasma insulin than FL birds (0.88 ng/ml).

The results for the plasma glucagon to insulin molar ratio (GTIR), APP, and free fatty acids (FFA) are presented in table 6.

GTIR- In Experiment 1, the GTIR was significantly affected by diet ($p=0.0378$), state ($p=0.0015$), and line x state ($p=0.0300$). Fat supplemented birds had a significantly lower average GTIR (6.7:1) than non-fat supplemented birds (8.9:1). Fasted birds had a significantly higher ratio (9.4:1) than did fed birds (6.2:1).

A line difference surfaced in the fasted state. That is, LL fasted chickens had a significantly higher ratio (11.2:1) than FL fasted ones (7.6:1). Fasted FL birds did not differ significantly from fed FL (6.6:1) and LL (5.9:1) birds.

APP- APP levels varied significantly with state ($p=0.0001$). Plasma APP levels were higher in fed (1.91 ng/ml) than fasted (0.92 ng/ml) chickens.

FFA- Only fasted birds were measured for FFA levels. FFA levels in the plasma were unaffected by line, diet, and sex.

Results for the within line partial correlations are shown in table 7.

FL

Glucose was significantly and positively correlated with APP ($r=0.25$; $p=0.0317$).

Glucagon was correlated with GTIR ($r=0.72$; $p=0.0001$) and PAF

(r=0.30; p=0.0077).

Insulin was negatively correlated with GTIR (r=-0.53; p=0.0001) and positively correlated with APP (r=0.34; p=0.0028).

GTIR was negatively correlated with APP (r=-0.26; p=0.0227).

LL

Glucose was positively correlated with plasma insulin (r=0.37; p=0.0009) and negatively with PAF (r=-0.24; p=0.0390).

Glucagon was negatively correlated with insulin (r=-0.31; p=0.0068) and positively with GTIR (r=0.63; p=0.0001).

Insulin was negatively correlated with GTIR (r=-0.53; p=0.0001) and positively correlated with APP (r=0.24; p=0.0395).

Table 5: The effect of line, dietary fat supplementation, state (fasted and fed), and sex on plasma levels of glucose, IRG, and IRI in 4 week old chicks (experiment 1).

		GLUCOSE (mg%)	IRG (ng/ml)	IRI (ng/ml)
LINE				
FL		228±4[a]	1.7±0.1[a]	0.76±0.1[a]
LL		251±4[b]	2.1±0.1[a]	0.73±0.1[a]
		(0.0004)	(0.0655)	(0.8182)
DIET				
FS		245±3[a]	1.8±0.1[a]	0.76±0.1[a]
NFS		234±3[b]	2.0±0.1[a]	0.73±0.1[a]
		(0.0265)	(0.1920)	(0.7709)
STATE				
FASTED		211±3[b]	1.8±0.1[a]	0.45±0.1[b]
FED		268±3[a]	2.0±0.1[a]	0.98±0.1[a]
		(0.0001)	(0.0843)	(0.0001)
SEX				
F		239±3[a]	1.9±0.1[a]	0.74±0.1[a]
M		239±3[a]	1.9±0.1[a]	0.68±0.1[a]
		(0.8491)	(0.9786)	(0.4403)
LINE X STATE				
FL	FASTED	204±5	1.5±0.2	0.52±0.1
FL	FED	253±5	1.9±0.2	0.88±0.1
LL	FASTED	219±5	2.0±0.2	0.38±0.1
LL	FED	282±5	2.2±0.2	1.07±0.1
		(0.1093)	(0.5288)	(0.0296)

Mean±sem for 92-96 chicks/main effect.
Mean±sem for 46-48 chicks/2 way interaction.
Numbers in parentheses indicate the probability of a greater F value.
[a,b] means for any treatment not having the same superscript are significantly different.
FS=fat-supplemented.
NFS=non-fat supplemented.

Table 6: The effect of line, dietary fat supplementation, state (fasted and fed), and sex on plasma levels of APP, and FFA's and on the circulating glucagon to insulin molar ratio (GTIR) in 4 week old chicks (experiment 1).

		GTIR	APP (ng/ml)	FFA (nmol/l)
LINE				
FL		7.1±0.8[a]	1.5±0.1[a]	970±58[a]
LL		8.6±0.9[a]	1.3±0.1[a]	1100±61[a]
		(0.2329)	(0.1756)	(0.2346)
DIET				
FS		6.7±0.7[b]	1.4±0.1[a]	1043±40[a]
NFS		8.9±0.7[a]	1.4±0.1[a]	1027±36[a]
		(0.0378)	(0.6804)	(0.7718)
STATE				
FASTED		9.4±0.7[a]	0.9±0.1[b]	1035±26
FED		6.2±0.7[b]	1.9±0.1[a]	----
		(0.0015)	(0.0001)	
SEX				
F		7.6±0.7[a]	1.4±0.1[a]	1041±34[a]
M		8.0±0.8[a]	1.4±0.1[a]	1029±41[a]
		(0.7121)	(0.8172)	(0.8389)
LINE X STATE				
FL	FASTED	7.6±1.1	1.2±0.2	970±58
FL	FED	6.6±1.0	1.9±0.2	----
LL	FASTED	11.2±1.1	0.7±0.2	1100±61
LL	FED	5.9±1.1	1.9±0.1	----
		(0.0300)	(0.1180)	(0.2346)

Mean±sem for 92-96 chicks/main effect.
Mean±sem for 46-48 chicks/2 way interaction.
Numbers in parentheses indicate the probability of a greater F value.
[a,b] means for any treatment not having the same superscript are significantly different.
FS=fat-supplemented.
NFS=non-fat supplemented.

Table 7: Partial correlation coefficients (r) among plasma glucose, glucagon (IRG), insulin (IRI), glucagon to insulin molar ratio (GTIR), APP, and percent abdominal fat (PAF) for 4 week old FL/LL chicks raised on fat and non-fat supplemented diets (experiment 1).

	IRG	IRI	GTIR	APP	PAF
FAT LINE					
GLUCOSE	0.077 (0.5076)	0.200 (0.0827)	-0.024 (0.8394)	0.247 (0.0317)	-0.022 (0.8512)
IRG		-0.163 (0.1596)	0.722 (0.0001)	-0.214 (0.0634)	0.303 (0.0077)
IRI			-0.533 (0.0001)	0.338 (0.0026)	-0.041 (0.7274)
GTIR				-0.261 (0.0227)	0.083 (0.4785)
APP					-0.185 (0.1087)
LEAN LINE					
GLUCOSE	-0.001 (0.9930)	0.371 (0.0009)	-0.136 (0.2399)	0.035 (0.7602)	-0.236 (0.0390)
IRG		-0.306 (0.0068)	0.631 (0.0001)	-0.204 (0.0751)	-0.082 (0.4796)
IRI			-0.528 (0.0001)	0.235 (0.0395)	-0.154 (0.1802)
GTIR				-0.098 (0.3956)	-0.027 (0.8143)
APP					-0.062 (0.5948)

The numbers in parentheses indicate the probability of a greater absolute value of "r".
Correlation for 90-96 birds per line.

EXPERIMENT 2

The results for plasma glucose, IRG, and IRI are presented in table 8.

GLUCOSE- Glucose levels were significantly affected by line ($p=0.0001$), temperature ($p=0.0001$), and state ($p=0.0001$). In addition, there was a significant temperature x state interaction ($p=0.0001$). FL plasma glucoses levels averaged 191 mg% to the LL's higher 213 mg%. Room temperature circulating glucose levels were higher (216 mg%) than cold room levels (187 mg%). Fasted birds had lower plasma glucoses (186 mg%) than fed birds (217 mg%). With respect to the temperature x state interaction, room temperature fed birds had a higher average plasma glucose level (245 mg%) than room temperature fasted (187 mg%) or cold room fasted (185 mg%) birds. Cold room "fed" birds also had lower (189 mg%) levels than the room temperature fed birds.

GLUCAGON- Plasma glucagon levels were affected by temperature ($p=0.0001$). In addition the interactions line x state ($p=0.0010$) and temperature x state ($p=0.0002$) were also highly significant. There was a trend towards significance for the line x temperature x state ($p=0.0678$). Cold room birds had a higher (3.6 ng/ml) average plasma glucagon than room temperature birds (1.8 ng/ml).

Cold room fed birds had a significantly higher average plasma glucagon level (4.0 ng/ml) in comparison to the other

three groups. Cold room fasted birds had a significantly higher average plasma glucagon (3.1 ng/ml) than room temperature fasted birds (2.4 ng/ml). In contrast to the other three groups, room temperature held fed birds had a significantly lower average plasma glucagon concentration (1.1 ng/ml).

Fasted FL birds had a significantly higher average plasma glucagon level (3.4 ng/ml) than fed birds of the same line (2.2 ng/ml). The opposite was seen in the LL where the fed birds had a significantly higher average (3.0 ng/ml) than the fasted birds (2.2 ng/ml).

INSULIN- Plasma insulin levels were significantly affected by temperature ($p=0.0001$), state ($p=0.0002$), temperature x state ($p=0.0001$), and sex ($p=0.0483$). Cold room exposed birds had a significantly lower plasma insulin (0.26 ng/ml) than room temperature birds (0.43 ng/ml). Fasting significantly lowered plasma insulin from an average of 0.43 ng/ml in the fed state to 0.26 ng/ml. The average plasma insulin level was significantly highest in room temperature-kept fed birds (0.65 ng/ml). The other three groups did not differ significantly from one another; cold room exposed fasted (0.31 ng/ml), fed (0.21 ng/ml), and room temperature-kept fasted (0.21 ng/ml). Females had significantly elevated average plasma insulins (0.39 ng/ml) compared to males (0.30 ng/ml).

Without PAF included as a covariate in the model, there was a significant effect of line (p=0.0253) on plasma insulin. FL birds had a significantly higher overall average of 0.39 ng/ml compared to the LL's average of 0.30 ng/ml. As PAF was not included in the model, this would suggest that the differences in percent abdominal fat did not account for the line differences in plasma insulin.

The plasma GTIR and FFA results are found in table 9.

GTIR- The ratio of glucagon to insulin (GTIR) was significantly affected by temperature (p=0.0001) and by a temperature x state interaction (p=0.0001). Cold room-exposed birds had an average ratio of 31.5:1 while room temperature birds had a ratio of only 13.6, less than half. Cold room fed birds had the highest average GTIR (42.5:1)- significantly higher than fasted cold room (20.4:1) or room temperature (23.6:1) birds, the latter two groups having significantly higher ratios than the room temperature kept fed birds (4.0:1).

FFA- Free fatty acid levels were significantly higher (p=0.0001) in cold room (1591 nmoles/l) than room temperature birds (983 nmoles/l). In addition, there was a significant temperature x state interaction (p=0.0134). Cold room exposed fasted (1522 nmoles/l) and fed (165[illegible] nmoles/l) birds had significantly higher concentrations than room temperature fasted (1091 nmoles/l) and fed (875 nmoles/l).

The latter two differed significantly from each other.

The within line partial correlations results are shown in table 10.

FL

Glucose was significantly and positively correlated with glucagon ($r=0.36$; $p=0.0519$) and insulin ($r=0.41$; $p=0.0269$).

Glucagon was significantly and positively correlated with insulin ($r=0.38$; $p=0.0403$) and GTIR ($r=0.57$; $P=0.0012$).

LL

Glucose was significantly and negatively correlated with insulin ($r=-0.36$; $p=0.0340$).

Glucagon was significantly and positively correlated with GTIR ($r=0.67$; $p=0.0001$).

Insulin was significantly and negatively correlated with GTIR ($r=-0.53$; $p=0.0012$).

Table 8: The effect of line, temperature (cold room vs.room temp), state (fasted and fed), and sex on plasma levels of glucose, IRG, and IRI in 5 week old chicks (experiment 2).

		GLUCOSE (mg%)	IRG (ng/ml)	IRI (ng/ml)
LINE				
FL		191±5[b]	2.8±0.3[a]	0.39±0.03[a]*
LL		213±5[a]	2.6±0.3[a]	0.30±0.03[b]
		(0.0001)	(0.7082)	(0.0253)
TEMP				
CR		187±3[b]	3.6±0.2[a]	0.26±0.03[b]
RT		216±3[a]	1.8±0.2[b]	0.43±0.03[a]
		(0.0001)	(0.0001)	(0.0001)
STATE				
FASTED		186±3[b]	2.8±0.2[a]	0.26±0.03[b]
FED		217±3[a]	2.6±0.2[a]	0.43±0.03[a]
		(0.0001)	(0.5383)	(0.0002)
SEX				
F		202±2[a]	2.8±0.2[a]	0.39±0.03[a]
M		202±2[a]	2.5±0.2[a]	0.30±0.03[b]
		(0.9810)	(0.3525)	(0.0483)
TEMP X STATE				
CR	FASTED	185±5	3.1±0.3	0.31±0.04
CR	FED	189±4	4.0±0.3	0.21±0.04
RT	FASTED	187±4	2.4±0.3	0.21±0.04
RT	FED	245±4	1.1±0.3	0.65±0.04
		(0.0001)	(0.0002)	(0.0001)
LINE X STATE				
FL	FASTED	174±6	3.4±0.4	0.31±0.05
FL	FED	208±6	2.2±0.4	0.44±0.05
LL	FASTED	198±6	2.2±0.4	0.21±0.05
LL	FED	227±6	3.0±0.4	0.41±0.05
		(0.6067)	(0.0013)	(0.4411)

continued next page

Table 8: cont'd

			GLUCOSE (mg%)	IRG (ng/ml)	IRI (ng/ml)
LINE X TEMP X STATE					
FL	CR	FA	166±7	3.9±0.5	0.40±0.07
FL	CR	FE	181±7	3.3±0.5	0.18±0.07
FL	RT	FA	180±7	2.8±0.5	0.23±0.07
FL	RT	FE	234±7	1.1±0.5	0.70±0.07
LL	CR	FA	202±7	2.3±0.4	0.23±0.06
LL	CR	FE	197±7	4.8±0.4	0.23±0.06
LL	RT	FA	193±8	2.0±0.5	0.19±0.06
LL	RT	FE	256±7	1.2±0.5	0.58±0.06
			(0.1248)	(0.0691)	(0.0651)

*=without PAF as a covariate in the model.
Mean±sem for 38-40 chicks/main effect.
Mean±sem for 19-20 chicks/2 way interaction.
Mean±sem for 9-10 chicks/3 way interaction.
Numbers in parentheses indicate the probability of a greater F value.
^{a,b} means for any treatment not having the same superscript are significantly different.
CR=cold room.
RT=room temperature.

Table 9: The effect of line, temperature (cold room vs.room temp.), state (fasted and fed), and sex on plasma levels of FFA's and on the circulating glucagon to insulin molar ratio (GTIR) in 5 week old chicks (experiment 2).

		GTIR	FFA (nmol/l)
LINE			
FL		20.4±2.4[a]	1309±75[a]
LL		24.9±2.2[a]	1264±76[a]
		(0.1713)	(0.7395)
TEMP			
CR		31.5±2.3[a]	1591±51[a]
RT		13.8±2.2[b]	981±50[b]
		(0.0001)	(0.0001)
STATE			
FASTED		22.0±2.3[a]	1306±52[a]
FED		23.3±2.2[a]	1267±48[a]
		(0.7021)	(0.5842)
SEX			
F		20.9±2.2[a]	1325±52[a]
M		24.4±2.4[a]	1247±54[a]
		(0.2156)	(0.3137)
TEMP X STATE			
CR	FASTED	20.4±3.5	1522±73
CR	FED	42.5±3.1	1662±69
RT	FASTED	23.6±3.1	1090±73
RT	FED	4.0±3.1	872±69
		(0.0001)	(0.0134)
LINE X STATE			
FL	FASTED	22.2±3.5	1344±88
FL	FED	18.6±3.2	1274±94
LL	FASTED	21.9±3.3	1267±94
LL	FED	28.0±3.0	1260±93
		(0.1289)	(0.6763)

Mean±sem for 38-40 chicks/main effect.
Mean±sem for 19-20 chicks/2 way interaction.
Numbers in parentheses indicate the prob.>F.
[a,b] means for any treatment not having the same superscript are significantly different.
CR=cold room. RT=room temperature.

Table 10: Partial correlation coefficients (r) among plasma glucose, glucagon (IRG), insulin (IRI), glucagon to insulin molar ratio (GTIR), free fatty acids (FFAs), and percent abdominal fat (PAF) for 5 week old FL/LL chicks raised on a commercial diet and exposed to the different temperatures (experiment 2).

	IRG	IRI	GTIR	FFA	PAF
FAT LINE					
GLUCOSE	0.364 (0.0519)	0.411 (0.0269)	0.165 (0.3925)	0.026 (0.8930)	0.100 (0.6028)
IRG		0.382 (0.0407)	0.574 (0.0012)	-0.178 (0.3553)	0.073 (0.7081)
IRI			-0.211 (0.2721)	-0.102 (0.5983)	0.027 (0.8887)
GTIR				-0.098 (0.6144)	0.209 (0.2766)
FFA					-0.052 (0.7900)
LEAN LINE					
GLUCOSE	0.058 (0.7450)	-0.364 (0.0340)	-0.077 (0.6654)	0.143 (0.4184)	-0.154 (0.3850)
IRG		-0.128 (0.4710)	0.670 (0.0001)	0.104 (0.5565)	-0.216 (0.2194)
IRI			-0.532 (0.0012)	-0.203 (0.2494)	0.079 (0.6560)
GTIR				0.215 (0.2215)	-0.022 (0.9031)
FFA					-0.002 (0.9910)

The numbers in parentheses indicate the probability of a greater absolute value of "r".
Correlation for 36-40 birds/line.

Experiment 2: analyzed without the cold room data-

The results for plasma glucose, IRG, and IRI are found in table 11.

GLUCOSE- There was a significant line effect on plasma glucose levels (p=0.0009). FL birds had a lower plasma glucose average (190 mg%) than LL birds (214 mg%). Plasma glucose levels were also affected by state (p=0.0001). Fasted birds had lower levels (186 mg%) than fed birds (218 mg%).

GLUCAGON- There was a significant line x state interaction (p=0.0143). FL fasted (3.0 ng/ml) birds had a significantly higher average than FL fed (1.8 ng/ml) birds. In the LL, fed and fasted birds did not differ significantly in plasma glucagon levels (3.4 and 2.6 ng/ml respectively). Fasted LL birds did not differ significantly from FL fed birds.

INSULIN- There were significant effects of state (p=0.0086), sex (p=0.0448), and line x sex (p=0.0440). FL females had a higher average plasma insulin level (0.53 ng/ml) than FL males (0.29 ng/ml) and LL females (0.28 ng/ml) and LL males (0.27 ng/ml).

The GTIR and FFA results are found in table 12.

GTIR- Interestingly, there was almost a significant effect of line (p=0.0595) on GTIR. LL birds had a higher ratio (30.6:1) than did FL birds (14.0:1).

FFA- There was no effect of treatment on FFA levels.

The within line partial correlations, without the cold room data, are found in table 13.

FL-

Glucose was significantly and positively correlated with insulin (r=0.72; p=0.0001) and negatively with GTIR (r=-0.48; p=0.0066) and FFA (r=-0.60; p=0.0005).

Glucagon was significantly and positively correlated with GTIR (r=0.70; p=0.0001).

Insulin was significantly and negatively correlated with both GTIR (r=-0.61; p=0.0003) and FFA (r=-0.48; p=0.0072).

GTIR was significantly and positively correlated with FFA (r=0.40; p=0.0297).

LL-

Glucose was significantly and negatively correlated with glucagon (r=-0.53; p=0.0009), GTIR (r=-0.60; p=0.0001), FFA (r=-0.35; p=0.0426), and PAF (r=-0.39; p=0.0215).

Glucagon was significantly and negatively correlated with insulin (r=-0.57; p=0.0003) and positively with GTIR (r=0.88; p=0.0001) and FFA (r=0.57; p=0.0004).

Insulin was significantly and negatively correlated with GTIR (r=-0.76; p=0.0001) and with FFA (r=-0.48; p=0.0033).

GIIR was significantly and positively correlated with FFA ($r=0.58$; $p=0.0003$).

Table 11: The effect of line, state (fasted and fed), and sex on plasma levels of glucose, IRG, and IRI in 5 week old chicks (experiment 2). **Cold room data excluded.**

		GLUCOSE (mg%)	IRG (ng/ml)	IRI (ng/ml)
LINE				
FL		190±5[b]	2.4±0.4[a]	0.41±0.06[a]
LL		214±5[a]	3.0±0.4[a]	0.28±0.06[a]
		(0.0009)	(0.3975)	(0.2133)
STATE				
FASTED		186±5[b]	2.8±0.3[a]	0.26±0.04[b]
FED		218±5[a]	2.6±0.3[a]	0.42±0.04[a]
		(0.0001)	(0.5706)	(0.0086)
SEX				
F		202±5[a]	2.8±0.3[a]	0.40±0.04[a]
M		201±5[a]	2.6±0.3[a]	0.28±0.04[b]
		(0.8941)	(0.7148)	(0.0448)
LINE X STATE				
FL	FASTED	178±8	3.0±0.5	0.34±0.07
FL	FED	213±8	1.8±0.5	0.47±0.07
LL	FASTED	194±9	2.6±0.5	0.18±0.07
LL	FED	223±8	3.4±0.5	0.37±0.07
		(0.6330)	(0.0143)	(0.6236)
LINE X SEX				
FL	F			0.53±0.08
FL	M			0.29±0.06
LL	F			0.28±0.06
LL	M			0.27±0.08
				(0.0440)

Mean±sem for 18-20 chicks/main effect.
Mean±sem for 9-10 chicks/2 way interaction.
Numbers in parentheses indicate the probability of a greater F value.
[a,b] means for any treatment not having the same superscript are significantly different.

Table 12: The effect of line, state (fasted and fed), and sex on plasma FFA levels and on the circulating glucagon to insulin molar ratio in 5 week old chicks fed a commercial diet (experiment 2). **Cold room data excluded.**

	GTIR	FFA (nmol/l)
LINE		
FL	14.0±5.1[b]	1227±110[a]
LL	30.6±4.8[a]	1335±115[a]
	(0.0595)	(0.5847)
STATE		
FASTED	21.3±3.6[a]	1296±81[a]
FED	23.2±3.2[a]	1265±71[a]
	(0.6843)	(0.7757)
SEX		
F	19.1±3.4[a]	1300±76[a]
M	25.5±3.6[a]	1261±80[a]
	(0.2122)	(0.7264)
LINE X STATE		
FL FASTED	15.7±6.1	1265±129
FL FED	12.2±6.3	1189±136
LL FASTED	26.9±6.0	1328±141
LL FED	34.3±5.7	1342±136
	(0.2662)	(0.6827)

Mean±sem for 18-20 chicks/main effect.
Mean±sem for 9-10 chicks/2 way interaction.
Numbers in parentheses indicate the probability of a greater F value.
[a,b] means for any treatment not having the same superscript are significantly different.

Table 13: Partial correlation coefficients (r) among plasma glucose, glucagon (IRG), insulin (IRI), glucagon to insulin molar ratio (GTIR), free fatty acids (FFA's), and percent abdominal fat (PAF) for 5 week old FL/LL chicks raised on a commercial diet (experiment 2). **Cold room data excluded.**

	IRG	IRI	GTIR	FFA	PAF
FAT LINE					
GLUCOSE	-0.273 (0.1438)	0.720 (0.0001)	-0.485 (0.0066)	-0.596 (0.0005)	-0.024 (0.9015)
IRG		-0.115 (0.5462)	0.693 (0.0001)	0.361 (0.1066)	0.129 (0.4953)
IRI			-0.612 (0.0003)	-0.481 (0.0072)	-0.035 (0.8557)
GTIR				0.397 (0.0297)	0.199 (0.2915)
FFA					0.041 (0.8290)
LEAN LINE					
GLUCOSE	-0.534 (0.0009)	0.271 (0.1153)	-0.600 (0.0001)	-0.345 (0.0426)	-0.387 (0.0215)
IRG		-0.572 (0.0003)	0.878 (0.0001)	0.568 (0.0004)	0.227 (0.1893)
IRI			-0.762 (0.0001)	-0.482 (0.0033)	-0.202 (0.2445)
GTIR				0.578 (0.0003)	0.316 (0.0644)
FFA					0.291 (0.0896)

The numbers in parentheses indicate the probability of a greater absolute value of "r".
Correlation for 17-20 birds per line.

EXPERIMENT 3

The glucose, IRG, and IRI results are shown in table 14.

GLUCOSE-Plasma glucose levels were significantly affected by line ($p=0.0519$) and state ($p=0.0001$). The FL averaged 222 mg% to the LL's average of 248 mg%. In descending order of magnitude were refed birds averaging 266 mg%, fed birds at 239 mg%, and fasted birds with a 200 mg% average plasma glucose concentration.

GLUCAGON- Plasma glucagon levels were affected significantly only by state ($p=0.0098$), being highest in fasted birds (1.9 ng/ml) followed by fed birds (1.4 ng/ml) and refed birds (1.0 ng/ml). Refed birds differed significantly from fasted birds but fed birds did not differ significantly from either.

INSULIN- Plasma insulin levels were also significantly affected by state ($p=0.0001$). Refed birds averaged 1.00 ng/ml while fed birds averaged 0.40 ng/ml and fasted birds averaged 0.18 ng/ml.

The GTIR and APP results are presented in table 15.

GTIR- The glucagon to insulin ratio was significantly altered in response to state ($p=0.0001$). Fasted birds possessed the highest average GTIR (25.4:1) significantly higher than the fed birds' 9.0:1 which was significantly higher than the refed birds' 2.1:1 ratio.

APP- In addition to insulin and glucagon, plasma APP concentrations were also significantly affected by state ($p=0.0001$). Refed birds exhibited the highest levels (6.3 ng/ml), fed birds the second highest (2.6 ng/ml), and fasted birds the lowest (1.0 ng/ml) in terms of plasma APP. All 3 states differing significantly from one another.

Results for the within line partial correlations are found in table 16.

FL

Glucagon was significantly and positively correlated with GTIR ($r=0.87$; $p=0.0001$). It was also correlated with PAF ($r=0.37$; $p=0.0680$) though non-significantly.

APP tended to be correlated with PAF ($r=0.35$; $p=0.0846$).

LL-

Glucose was significantly and positively correlated with insulin ($r=0.55$; $p=0.0093$) and negatively with APP ($r=-0.48$; $p=0.0282$).

Glucagon was significantly and negatively correlated with insulin ($r=-0.46$; $p=0.0362$) and PAF ($r=-0.58$; $p=0.0057$) and positively with GTIR ($r=0.80$; $p=0.0001$).

Insulin was significantly and negatively correlated with APP ($r=-0.45$; $p=0.0403$) and positively with PAF ($r=0.44$; $p=0.0484$).

GIIR was significantly and negatively correlated with PAF ($r = -0.49$; $p = 0.0247$).

Table 14: The effect of line, state (fasted (16 hr) vs. fed vs. fasted for 16 hr then refed for 45 min), and sex on plasma levels of glucose, IRG, and IRI in 5 week old chicks fed a commercial diet (experiment 3).

	GLUCOSE (mg%)	IRG (ng/ml)	IRI (ng/ml)
LINE			
FL	222±8^{b}	1.5±0.2^{a}	0.48±0.05^{a}
LL	248±8^{a}	1.4±0.2^{a}	0.57±0.06^{a}
	(0.0519)	(0.7526)	(0.3709)
STATE			
FASTED	201±7^{c}	1.9±0.2^{a}	0.18±0.05^{c}
FED	239±7^{b}	1.4±0.2^{ab}	0.40±0.05^{b}
REFED	266±7^{a}	1.0±0.2^{b}	1.00±0.05^{a}
	(0.0001)	(0.0098)	(0.0001)
SEX			
F	235±6^{a}	1.6±0.2^{a}	0.49±0.04^{a}
M	236±6^{a}	1.3±0.2^{a}	0.56±0.04^{a}
	(0.9526)	(0.2034)	(0.2161)
LINE X STATE			
FL FASTED	180±11	2.3±0.3	0.12±0.08
FL FED	237±10	1.2±0.3	0.42±0.07
FL REFED	251±12	1.0±0.3	0.92±0.09
LL FASTED	221±11	1.5±0.3	0.25±0.08
LL FED	242±10	1.6±0.3	0.39±0.07
LL REFED	282±12	1.0±0.3	1.07±0.09
	(0.1803)	(0.1157)	(0.4174)

Mean±sem for 28-30 chicks/main effect.
Mean±sem for 14-15 chicks/2 way interaction.
Numbers in parentheses indicate the probability of a greater F value.
^{a,b} means for any treatment not having the same superscript are significantly different.

Table 15: The effect of line, state (fasted (16 hr) vs. fed vs. fasted for 16 hr then refed for 45 min), and sex on plasma APP levels and on the circulating glucagon to insulin molar ratio in 5 week old chicks fed a commercial diet (experiment 3).

		GTIR	APP (ng/ml)
LINE			
FL		12.2±2.0[a]	3.5±0.4a
LL		12.1±2.1[a]	3.1±0.4[a]
		(0.8374)	(0.6065)
STATE			
FASTED		25.4±2.6[a]	1.0±0.4[c]
FED		9.0±2.6[b]	2.6±0.4[b]
REFED		2.1±2.5[c]	6.3±0.4[a]
		(0.0001)	(0.0001)
SEX			
F		13.5±2.1[a]	3.3± 0.3[a]
M		10.9±2.0[a]	3.3±0.3[a]
		(0.3703)	(0.9150)
LINE X STATE			
FL	FASTED	29.7±3.6	0.9±0.6
FL	FED	5.0±3.5	2.8±0.5
FL	REFED	1.9±3.4	6.7±0.7
LL	FASTED	21.2±3.6	1.0±0.6
LL	FED	13.0±3.9	2.3±0.5
LL	REFED	2.3±3.6	6.0±0.6
		(0.0919)	(0.7005)

Mean±sem for 28-30 chicks/main effect.
Mean±sem for 14-15 chicks/2 way interaction.
Numbers in parentheses indicate the probability of a greater F value.
[a,b] means for any treatment not having the same superscript are significantly different.

Table 16: Partial correlation coefficients (r) among plasma glucose, glucagon (IRG), insulin (IRI), glucagon to insulin molar ratio (GTIR), APP, and percent abdominal fat (PAF) for 5 week old FL/LL chicks raised on a commercial diet (experiment 3).

	IRG	IRI	GTIR	APP	PAF
FAT LINE					
GLUCOSE	0.077 (0.7134)	-0.009 (0.9668)	0.161 (0.4433)	0.103 (0.6253)	0.298 (0.1477)
IRG		-0.090 (0.6673)	0.872 (0.0001)	0.173 (0.4091)	0.371 (0.0680)
IRI			-0.242 (0.2431)	-0.129 (0.5396)	0.116 (0.5824)
GTIR				0.184 (0.3779)	0.275 (0.1836)
APP					0.352 (0.0846)
LEAN LINE					
GLUCOSE	-0.356 (0.1129)	0.553 (0.0093)	-0.303 (0.1824)	-0.478 (0.0282)	0.250 (0.2748)
IRG		-0.459 (0.0362)	0.798 (0.0001)	0.134 (0.5611)	-0.581 (0.0057)
IRI			-0.398 (0.0737)	-0.451 (0.0403)	0.436 (0.0484)
GTIR				-0.081 (0.7270)	-0.488 (0.0247)
APP					-0.188 (0.4146)

The numbers in parentheses indicate the probability of a greater absolute value of "r".
Correlation for 27-30 birds per line.

DISCUSSION

These F6 generation FL/LL birds were seen to have maintained their respective differential abdominal fat percentages in all 3 experiments. The FL birds had 2 to 3 times the percent abdominal fat found in the LL birds, a fact which confirmed earlier work (50,8,9).

Experiment 1

Fasting had the well-known effect of lowering plasma glucose levels in both lines relative to feeding. It has been established that the initial response to fasting is a decrease in liver glycogen to one-tenth or less of normal fed levels during the first 24-36 hours (12). As glycogen is mobilized there is an increase in the activity of the gluconeogenic enzymes at the expense of protein stores and lipid depots. There is a rate differential, as more glucose is being used up than can be supplied by the body.

Glucose results agree with those of Simon and LeClercq (8) and Touchburn et al. (9). That is, irrespective of treatment FL birds had a significantly lower plasma glucose average than the LL birds. The fact that plasma insulin levels did not vary with line, would lead one to believe that the FL chickens were more sensitive to insulin than the LL. That is, for a given plasma insulin concentration, the FL was able to take up glucose (or inhibit glucose production) more efficiently than the LL. Regarding the line x state

interaction for insulin, in the fed state the FL had a lower plasma insulin level coupled with a lower glucose level than the LL and this would further support the FL's being more sensitive to plasma insulin levels.

In this experiment fat-suppplementation, while not altering absolute hormone or FFA levels, did significantly raise plasma glucose levels across all treatments. IRI sensitivity may have been decreased by fat feeding, or perhaps the dietary fat was used directly as an energy source, thus having a sparing effect on glucose. With (7%) added fat in the diet and less corn starch and D-glucose, the GTIR was found to be significantly lower than without added fat and this was surprising as a decreased GTIR would favour increased glucose utilization and a decreased circulating glucose level (depending on net flux i.e. glucose uptake over production). These observations appear contrary to those found in mammals. Perhaps this difference in hormone ratios was on the border of physiological significance and did not, in this case, represent a true dietary difference.

It has been reported that, in mammals, isocaloric diets low in carbohydrate produce a chronic elevation of basal glucagon levels and a reduction in insulin levels. Also, it is claimed that a high level of carbohydrate in the diet lowers plasma glucagon levels and raises plasma insulin levels (123). A similar situation is observed with a high protein diet. It is essential for the A- and B-cells to be

able to respond to dietary variations in order to assure proper energy maintenance. It has also been shown that the absorption of long-chain triglycerides following fat ingestion stimulates glucagon secretion via the enteric hormone release in mammals. This stimulation is however dependent on circulating glucose levels (123).

Although non-significantly different, there was a tendency for plasma IRG concentrations to be higher in the LL vs. the FL. This tendency might indicate that the LL birds were breaking down adipose tissue stores at a faster rate than the FL, resulting in less net fat being deposited in the LL. Plasma glucagon levels seemed to be unaffected by diet or state (fed or fasted). However, this latter observation may have been due to the fact that single blood samples were taken. Time course studies might have revealed line differences in plasma glucagon.

The GTIR was significantly higher in fasted vs. fed birds. This was not surprising for in the fasted state, the bird is not digesting and assimilating food from the gut and instead is relying on stored energy reserves to satisfy its energy demands. A higher glucagon to insulin ratio would result in greater lipolysis, glycogenolysis, and gluconeogenesis and in less glycogenesis and lipogenesis. Lipogenesis occurs at a faster rate in the FL (50). The fact that in the fasted state the LL birds had a significantly higher GTIR relative to the FL would further support the notion that LL birds

have lipolysis going on at a faster rate and thus deposit less net fat over the long run.

APP levels were affected by state with fed levels averaging 1.9 ng/ml compared to fasted levels averaging 0.9 ng/ml. These results are in keeping with those in the literature i.e. feeding elevates APP levels over fasted levels (79). There was a tendency for the FL birds to respond with a lesser APP release with respect to feeding over fasting (1.9 ng/ml compared to 1.2ng/ml) compared to the LL (1.9 ng/ml compared to 0.7 ng/ml). Perhaps normal circulating APP levels were higher in the FL overall. Simon (1984 pers. comm.) showed a significantly greater APP release in the FL at 8 minutes following refeeding at which time plasma levels plateaued. As APP is thought to be anti-lipolytic, these observations support the theory that less fat is being mobilized from fat stores (and not being redeposited) in the FL than in the LL and this in turn is resulting in more net fat deposition in the FL. These observations would suggest that if APP is a satiety factor (86) in the bird, as it might be in congenitally obese mice (81) that the FL birds have a less responsive satiety centre relative to the LL. As there is little, if any, difference in feed intake between the two lines, the satiety factor theory is not supported here.

It was also observed that APP concentrations were lower in both states relative to values quoted in the literature.

This latter observation might be ascribed to species differences (Kimmel, 1984 pers. comm.).

Fasted FFA levels were not affected by line or diet. However, differences in FFA's may not have been observed because of the single time sampling nature of this experiment. Relative rates of change in concentration cannot be estimated for any of the parameters in question. Had samples been drawn at different times, FFA differences might have been observed.

The addition of fat to the diets resulted in a slight increase in feed consumption in both lines (results not shown). Presumably fat improved the feed's palatability and thus increased feed intake. The birds that consumed the fat supplemented diet had a lower percent abdominal fat content. Fat in the feed may have lowered the rate of lipogenesis. In the LL, as in the FL, females produced more abdominal fat than males. That females produce more fat than males is well documented. In chickens, estrogen increases fat deposition and thus explains the higher fat content of females (130,131).

Partial correlations were run to determine whether any consistencies or inconsistencies between the two lines and within each line existed with respect to the blood parameters measured and percent abdominal fat. Also it was of interest to confirm the classically held views that, for

example, insulin and glucagon are negatively correlated. The significant correlations would not, of course, indicate cause and effect.

The pattern between the FL and LL differed somewhat. For example, plasma glucose levels in both lines were not significantly correlated with plasma glucagon and only in the LL was glucose significantly correlated with insulin although a similar tendency was observed in the FL. There was a trend for plasma glucose to vary directly with APP in both lines, but the correlation was significant in the FL only. With only this information at hand, a clear interpretation of these results is not possible.

In the LL, glucose also varied indirectly with PAF indicating that, within this line, the fatter the bird the lower the plasma glucose level. This latter observation was in keeping with our initial hypothesis.

Plasma glucagon varied indirectly with plasma insulin in the LL. This was not the case in the FL. As these two hormones are known to work in opposition to each other by virtue of their respective responsiveness to glucose (92,59) it might be that a disorder in the regulation system exists between the A and B cells in the FL.

In both lines, plasma glucagon tended to vary indirectly with APP. This was not surprising as most knowledge, to date, indicates that these two hormones might work in

opposition to one another at physiological levels (81,46,23) though this is unproven. The direct relationship between insulin and APP was significant in both lines perhaps indicating that the two work in the same direction towards, for example, increased lipogenesis as suggested by others (81,48,23). Finally, in the FL only, was GTIR significantly and indirectly related to APP, further indicating a possible opposing action to glucagon.

In the FL glucagon levels varied directly with PAF. Therefore, in the FL, the fatter the bird, the higher the plasma glucagon but not necessarily the GTIR. This observation was contrary to our hypothesis that the FL birds would have lower plasma glucagon levels relative to the LL. It may not be ruled out, though, that the fatter FL birds were less sensitive to plasma glucagon's action.

Experiment 2

As in Experiment 1, plasma glucose levels were lower in the FL (190 mg%) than the LL (214 mg%) over all treatments. The cold room treatment had the effect of lowering plasma glucose levels as did fasting. This would agree with the observation that cold exposure increases glucose turnover, often resulting in a decrease in plasma glucose levels (124).

It was observed that "fed" cold room birds had plasma glucose levels similar to fasted room temperature birds and cold room fasted birds. This was not surprising as these "fed" birds were fasted during their 2-3 hour cold room exposure. The cold room treatment did not lower plasma glucose levels any more so than regular fasting. The cold temperature forced the birds to use up glucose to maintain normal body temperatures.

Plasma glucagon levels were raised upon cold exposure. This was expected as the birds would need to draw upon their adipose tissue reserves to provide FFA's for maintenance and, increased plasma glucagon would also provide glucose via its gluconeogenic/glycogenolytic actions. Also, it is known that circulating glucagon concentrations are elevated in response to stress in mammals (132) and this may have accentuated the response upon cold exposure.

Cold room "fed" birds had the highest glucagon level

indicating the more immediate perturbation to their systems for they had not even adjusted to the stress of fasting at the time they were put in the cold room. The increased plasma glucagon might be a first phase reaction to the effect of stress. During stress, there is an adrenergic enhancement of glucagon secretion and reduction of insulin release (132). In the fasted state, the cold room birds had a higher glucagon level than the room temperature fasted birds as the latter were not as stressed. It is believed that this is the first demonstration, in birds, that cold stress has the effect of increasing both plasma glucagon levels and the GTIR.

The line x state interaction, with repect to glucagon, presented an interesting situation. While fasted FL birds had a higher glucagon level than fed birds as expected, the opposite was found in the LL, that is, fed birds had higher levels than fasted birds. This was perhaps due to the confounding effect of the cold room treatment. Cold room exposure of "fed" LL birds gave a greater IRG release than in FL birds (4.8 vs. 3.3 ng/ml respectively) while in cold room fasted LL birds the response was less than in FL birds (2.3 vs. 3.3 ng/ml respectively), again, contrary to our hypothesis . Thus, averaged over all treatments, an apparent reversal could appear in the LL. The stress effect may not have been as severe in LL fasted birds as in fed birds when cold exposed as compared to the FL.

Without PAF as a covariate in the model equation, insulin was, for the first time, affected by line. FL birds had a significantly higher level than LL birds which would perhaps contribute to the greater rate of lipogenesis in the FL. The fact that PAF was not in the model would suggest that differences in abdominal fat did not account for the line differences in insulin. Previous work in other laboratories (8,9) had shown a significant line difference in this hormone only after an oral challenge. Therefore, further speculation at this time would be inappropriate. This may have been only a cold room effect.

The effect of state on plasma insulin levels was expected as was the effect of the cold room. Fasting and cold temperatures were expected to lower plasma insulin via sympathetic action (124) as dietary glucose levels were at a low. Room temperature fed birds had the highest level of plasma insulin as they were the only birds truly assimilating ingested glucose. Females were seen to have a higher insulin level than males, contributing to the increased lipogenesis and fat deposition in the females. This last observation was not consistent in the other 2 experiments and may thus have been only on the border of physiological significance.

Cold room exposure was expected to raise plasma glucagon levels and decrease insulin in order to increase gluconeogenesis, glycogenolysis, and lipolysis in order to

provide energy. The GTIR was highest in cold room "fed" birds showing their increased stress response while fasted birds did not differ from each other. The unstressed ad libitum fed birds had a dramatically reduced GTIR in comparison. When absolute levels of each hormone are kept in mind, it is this molar ratio of glucagon to insulin that best describes the events occuring.

No line differences were evident in FFA levels. Fasted room temperature birds, because of the lack of dietary glucose, liberated more FFA's than fed room temperature birds. In response to the cold room treatment, the birds dramatically elevated their FFA levels about two-fold normal fed levels in an attempt to maintain normal body temperature. The "fed" birds had the highest FFA concentrations due to their high GTIR and most probably a greater rate of lipolysis. The birds did not noticeably shiver in the cold room implying that non-shivering thermogenesis was occurring. Plasma FFA, which usually account for about 5% of total plasma lipids, are of major metabolic importance in mammals and birds (133). Studies have confirmed that plasma FFA concentrations are low in fed animals (200-300 nmoles/l), and are raised (500-1500 nmoles/l) during starvation, the major source of plasma FFA being adipose tissue (133).

The cold room, and the metabolic changes it induced, provided few significant correlations to discuss. In the FL, glucose varied directly with plasma glucagon and plasma

insulin. Insulin levels do rise with a rise in plasma glucose (124,17) but the response of glucagon most likely was due to the effect of cold.

In the LL, plasma glucose varied indirectly with circulating insulin levels. The reason for this relationship is uncertain and was probably due to the cold temperature. It may have been caused by an indirect inhibition of insulin release by sympathetic activation of the B cell by alpha-adrenergic receptors (124).

When the data were analyzed without the cold room data the correlations were more easily interpreted.

Experiment 2 without the cold room data

As in Experiment 1, plasma glucose levels were significantly lower in the FL than in the LL. Average glucose levels were 20-40% lower in this experiment versus Experiment 1. This difference may be accounted for by the change in diet i.e. from a semi-purified diet in Experiment 1 to a commercial diet in Experiment 2. Fasted birds displayed the classically lower plasma glucose levels relative to fed birds.

There was no significant difference between lines in terms of plasma glucagon in this experiment yet the tendency was for the LL to have a higher average level than the FL. FL birds displayed a significantly greater difference between fed and fasted glucagon levels than did the LL. The LL displayed higher fed levels of plasma glucagon as per our

hypothesis. As these chickens were rarely in the fasted state, by virtue of their crop and thus a constant metering of nutrients, perhaps this increased plasma glucagon could have led to increased lipolysis and decreased lipogenesis in the LL.

In terms of plasma insulin, fasting reduced insulin levels and while there was no line effect (therefore the line effect above was probably due to the cold) there were significant sex and line x sex effects. Females exhibited a higher average compared to males. FL females averaged significantly higher insulin levels than FL males and also LL females and males. This increased insulin, coupled with higher estrogen levels (130,131), may have been a factor in the increased PAF of the FL females.

It was observed that, overall, plasma IRG levels were slightly higher and IRI levels slightly lower in Experiment 2 versus Experiment 1. Differences in the type of diet most probably explain these differences (123).

The GTIR was significantly higher in the LL relative to the FL (if $p=0.0595$ is accepted as significant). As explained in Experiment 1 this difference might lead to an overall tendency towards excessive fat deposition in the FL. As FFA levels did not vary between lines as might have been expected with such a different GTIR, it may have been that the rate flux hid what was really happening and time course

studies would have shown this.

The patterns of correlations were similar between lines.

Glucose varied indirectly with glucagon in both lines as expected but the relationship was significant only in the LL. In addition, while glucose and insulin varied directly in both lines, the relationship was significant only in the FL. In both lines, however, the GTIR was strongly and indirectly associated with plasma glucose levels. As the GTIR is the major factor regulating circulating glucose levels (77) this result was not surprising.

Plasma glucose also varied significantly and indirectly with plasma FFA's in both lines. The latter, in turn, varied directly with GTIR in both lines, further supporting the lipolytic action of glucagon and/or the anti-lipolytic action of insulin.

While FFA's tended to vary directly with glucagon in both lines, this relationship was significant only in the LL. In both lines, FFA's varied indirectly and significantly with plasma insulin and this would support the anti-lipolytic action of insulin (14).

Insulin and glucagon were negatively correlated in the LL but not in the FL as in Experiment 1. Thus it would appear that the regulation between insulin and glucagon is normal only in the LL.

Again it was noted that a significant relationship existed between glucose and PAF in the LL and that this relationship was negative. The greater the plasma glucose in the LL the lower the PAF. This would appear to agree with our hypothesis of leaner birds having a greater plasma glucose level. The fact that such was not the case in the FL would suggest that selection, within this line, altered the glucose to PAF relationship in a different way.

Experiment 3

As in previous experiments (8,9), plasma glucose was lower in the FL relative to the LL. Differences in plasma glucose were also observed with state, being highest in refed birds followed by fed then fasted birds. This was not surprising as it is well established that, immediately following ingestion of a meal, plasma glucose levels rise as a result of the absorption of incoming nutrients. The plasma level then decreases as insulin facilitates the uptake of dietary glucose by the liver and other cells. A 16 hr fast results in a drop in glucose levels and the body then relies more on non-dietary glucose i.e. glucose manufactured or released by the body to satisfy its glucose requirements.

Plasma glucagon levels varied with state in this trial. Levels were highest in fasted birds followed by fed then refed birds. The differences, however, reached significant levels only between the fasted and refed birds. Again, this was expected as in refed birds there was the least need for gluconeogenesis, glycogenolysis, and lipolysis whereas, in fasted birds, the need for all three was greatest. Therfore, in the fasted state, a greater circulating glucagon level was expected and observed.

In this experiment, as in the previous two, plasma insulin levels varied significantly with state. Insulin levels varied directly with glucose levels. A higher glucose concentration in response to refeeding triggered a

correspondingly higher plasma insulin concentration. As mentioned previously, during alimentation, the role of the islets is to prevent excessive increases in the plasma levels of the ingested nutrients, and, in particular, glucose (16).

The GTIR responded to the various states imposed as expected. That is, fasting elicited the highest GTIR followed by feeding then refeeding. Refeeding resulted in the lowest GTIR as insulin release was highly stimulated and glucagon release inhibited by the high dietary plasma glucose levels.

Recent observations have shown that a meal causes much larger rises in APP in young versus older (and presumably fatter) hens but not in males (Kimmel, 1984 pers. comm.). It was thus of greater interest to see whether APP differences existed between FL and LL chickens. When chickens were fasted for 16 hours then allowed to feed for 45 minutes the release of APP was highly stimulated - even over normal fed levels (Kimmel, pers. comm.). This was corroborated by the Experiment 3 results. Refed birds averaged an APP concentration of 6.3 ng/ml while fed birds averaged 2.6 ng/ml and fasted birds averaged 1.6 ng/ml. Again, as mentioned in Experiment 1, the lower APP concentrations found in the FL/LL chickens relative to the literature values might have been due to species differences. Unfortunately, no line differences in APP were apparent.

In the FL, the correlations that were established in the previous two experiments were not evident. It was noted though that APP tended to be correlated to PAF which would agree with previous observations.

In the LL, glucose varied directly with insulin and indirectly with APP. The first observation showed that, under normal circumstances, as in Experiement 1, IRI varies directly with glucose. The latter observation was inconsistent with respect to Experiment 1 where APP did not vary significantly with glucose.

LL plasma glucagon again varied inversely with insulin while in the FL this was not the case. This observation has consistently been made, save for the cold room analysis, in all 3 trials and perhaps represents a true impairment of the regulatory relationship between the A and B cells in the FL.

Insulin, in the LL, varied indirectly with APP and directly with PAF. The former observation was inconsistent with Experiment 1 results where insulin varied directly with APP. APP might be quite sensitive to dietary changes or the overall average might have been influenced by the different feeding states imposed between the 2 experiments. The observation that insulin varied directly with PAF was consistent with the work of others (8,9) in that higher plasma insulin levels could account for increased adiposity.

SUMMARY AND CONCLUSIONS

Four and five week old genetically fat (FL) and lean (LL) line chickens were studied to further examine the role of the endocrine pancreas on intermediary metabolism in relation to adiposity in birds. It was known that the FL had a lower plasma glucose level than the LL and that its adiposity was due, in part, to a glucose-insulin imbalance. In this research it was undertaken to also determine whether line differences in glucagon (IRG) and avian pancreatic polypeptide (APP), in addition to insulin (IRI), could help account for the differential fatness.

In vivo experiments, that were known to alter metabolism in general, were designed to try and bring out any line differences in hormonal levels. Treatments included different states (fed, fasted 16 hr, and fasted then re-fed), ambient temperatures (5°C and 22°C) and diets.

Fasting reduced glycemia and, while IRI levels decreased, IRG increases were inconsistent. A rise in the glucagon to insulin molar ratio (GTIR) suggested a conversion to a catabolic state. APP levels were highest in re-fed birds (6 times fasted levels) versus fed (2-3 times fasted levels). Though hypothesized to be lipogenic, IRI and APP levels were not elevated in the FL over the LL.

In response to cold, glycemia dropped by 40 mg% and FFA's rose and were associated with a rise in IRG and a decrease

in IRI in both lines as a catabolic state induced the mobilization of stored energy. Again, no line differences were evident.

Dietary fat supplementation increased plasma glucose levels with no change in plasma IRI, IRG, or FFA suggesting that the increased fat ingestion might spare glucose supplies.

The fact that plasma glucoses were significantly lower in the FL versus LL birds when plasma IRI, IRG and FFA's were the same would suggest in the FL either 1) an increased sensitivity to IRI resulting in a) increased synthesis of fat from glucose; b) increased deposition of fat from circulating triglyceride or c) decreased lipolysis of stored fat or 2) a decreased sensitivity to IRG resulting in decreased lipolysis of stored fat. There could also be a defect with respect to down regulation with respect to IRI.

While absolute plasma IRG or IRI concentrations often provided insight as to what was occuring metabolically, in cases where either or both hormone levels did not change significantly, it was the GTIR that helped provide an explanation. In one case in particular, the LL had a significantly greater GTIR than the FL. An increased GTIR was believed to be associated with increased gluconeogenesis, and glycogenolysis and decreased lipogenesis in the liver, and increased lipolysis in the adipose resulting in the net liberation of fat stores and thus a leaner bird.

It was also observed that IRI and IRG were not negatively correlated in the FL as normal metabolism would usually dictate. In the FL therefore, an abnormal relationship between these two hormones was suggested.

This research revealed no line differences in the circulating levels of these three pancreatic hormones. Future studies involving: 1) oral challenges 2) exogenous hormone challenges and 3) in vitro hormone action might reveal line differences.

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