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ABSTRACT

The glucoregulatory response to hypoglycemia in diabetic dogs was studied. Plasma glucose concentration was decreased in alloxan-diabetic, pancreatectomized and methylprednisolone (MP) treated dogs by an infusion of either phlorizin (PHL) or insulin. The plasma concentrations of glucose (PG), free fatty acids, catecholamines (in alloxan-diabetics only), immunoreactive glucagon (IRG) and insulin (IRI) were followed. Hepatic glucose production (Ra) and the overall rate of glucose disappearance from the circulation were calculated by tracer methods.

Insulin deprived diabetic dogs did not raise their Ra when exposed to either a phlorizin- or an insulin-induced decrease in PG. A PHL-induced drop in PG did not cause a reproducible increase of the already elevated plasma IRG. However, PHL in the pancreatectomized dogs brought about an increase of plasma IRG. Insulin caused plasma IRG concentration to decrease in the alloxan-diabetic dogs, while IRG was maintained constant in pancreatectomized dogs. Plasma epinephrine increased in the alloxan-diabetic dogs during the insulin infusion. Insulin decreased Ra in both groups of insulin deprived diabetic dogs.

A one week long insulin treatment restored glucoregulation in alloxan diabetic dogs subjected to a PHL infusion.

MP treatment raised basal Ra to a diabetic level, while PG and IRG were maintained in the normal range, and insulin was slightly elevated. Both insulin- and PHL-induced drops of PG brought about an increase in Ra in the MP treated dogs. A heighten sensitivity of the glucoregulatory mechanism was

observed in these animals.

Conclusions: 1) The absence of a glucoregulatory response to hypoglycemia in insulin deprived diabetic dogs is due (a) to an insensitivity of the diabetic liver to glucagon and to epinephrine, (b) to the inhibition by insulin of hepatic glucose production and glucagon release, hypoglycemia is insulin-induced. 2) Elevated plasma IRI and the effects of methylprednisolone results in an enhanced sensitivity of the glucoregulatory mechanism.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. G. Hetenyi under whose supervision this work was conducted. His patient guidance, encouragements and understanding as well as his judicious use of Occam's razor were invaluable.

Many thanks to Dr. M. Vranic, University of Toronto, for the technical assistance of his laboratory for IRI, IRG, and catecholamine determinations.

Special thanks to Dr.M. Keaney, D.V.M., Director of the Animal Care Services of the University of Ottawa, for initiating me to the complicated business of caring for a sick animal, as well as for her friendship and moral support.

I must thank Mrs. C. Ferrarotto, Miss. M. Byers, Mr. J. Anthony and Mr. R. Layberry for their excellent technical assistance and their willingness to share some of the trade secrets of laboratory and animal work.

The technical assistance of Mrs. N. Kovacevic, P.Eng. and Mrs. D. Bilinski, in Toronto, was greatly appreciated.

The surgical expertise of Mrs. M. Bosc-Davie and her staff was indispensible to the second series of experiments and was greatly appreciated.

I wish to extend my thanks to the staff of the Animal Care Services, and especially to Mr. M. Marchand, for their ceaseless

concern for, and care of the diabetic dogs used in this study.

I am much obliged to Miss. E. McNally for graciously accepting to type parts of this manuscript.

It is with pleasure that I acknowledge the finacial support of the Medical Research Council of Canada. Without their support this work could not have been carried out.

Finally, I also wish to acknowledge the generous finacinal aid from the Ministry of Colleges and Universities of the province of Ontario, in the form of an Ontario Graduate Scholarship which I was awarded for the years 1982-83 and 1983-84.

B.T.L.

Edmonton, Alberta
April 8th, 1984

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CHAPTER I

INTRODUCTION

I General Introduction

The rise in plasma glucagon concentration, in patients with type I diabetes (IDDM) (Benson et al., 1977; Drost et al., 1980; Hilsted et al., 1981; Kleinbaum et al., 1983 and Sacca et al., 1981), streptozotocin diabetic rats (Patel 1983b) and alloxan-diabetic dogs (Braaten et al., 1974), when subjected to insulin-induced hypoglycemia, is either decreased (Braaten et al., 1974) or unchanged (Drost et al. 1980 and Sacca et al., 1979) or increased, but substantially less than observed in nondiabetic control subjects (Benson et al.,1977; Histed et al., 1981; Kleinbaum et al., 1983 and Patel, 1983 a,b). Catecholamines are reported to be released either in smaller amounts (Kleinbaum et al., 1983 and Patel, 1983 a,b) or much later (Sacca et al., 1979) than in appropriate controls. Plasma glucose concentration is decreased to a further extent, and either remains low or returns to basal level slower than in control.

In the light of these seemingly paradoxical observations, one may ask if the blunted glucagon response is due to hyperinsulinemia or to diabetes. One may also ask whether the blunted glucagon release and glucoregulatory responses were due to

permanently altered metabolism inherent in diabetes, or to the bihormonal abnormality observed in diabetes (i.e. high glucagon and low insulin concentration in the plasma) which could be resolved by proper treatment.

II Glucoregulation

All tissues in the body use glucose as an energy yielding substrate. A few tissues such as the central nervous system (CNS), the renal medulla, and the erythrocytes, can use only glucose as a source of energy. Other tissues also use other fuels, mainly fatty acids and ketone bodies. The brain may also use ketone bodies, however, in the fed state these compounds are present in small amounts in the circulation. Since survival depends on the proper functioning of the CNS, which in turn is dependent on an adequate energy supply, it is understandable that the plasma glucose concentration is regulated and maintained near a level which is adequate for the CNS.

This may be illustrated by the fact that if the glucose concentration is decreased, for example by strenuous exercise, it is soon returned to its pre-perturbation level as a result of changes in the rate of glucose appearance (Ra) and disappearance (Rd) from the circulating glucose pool. For such a system to work rapidly and efficiently it would have to be made up of a glucose producer, a glucose store and a rapid

input output controlling device. The store and producer is the liver while the controlling device is a neuro-hormonal system which acts on the liver and responds to changes in the concentration of plasma glucose.

Glucose is stored in the liver as glycogen - a branched polymer of glucose - by a process know as glycogenesis. The glucose residues are either linked by the α-1,4-glycosidic or the α-1,6-glycosidic bond. The latter is responsible for branching. As a first step in the process, glucose is transformed to glucose-6-phosphate by the enzyme glucokinase. Consequently glucose-6-phosphate is converted to glucose-1-phosphate by the reversible enzyme phosphoglucomutase. The glucose-1-phosphate is then activated by combining with uridine triphosphate (UTP) producing uridine diphosphate glucose (UDPG). It is this activated molecule which interacts with the hydroxyl group of carbon 4 of glucose at the end of the glycogen molecule, via the catalytic action of glycogen synthetase I. This results in the addition of a new residue to the polymer. Branching enzyme catalyses the formation of the α-1,6 bond between UDPG and glucose residues in the glycogen chain.

Glucose-6-phosphate is an intermediate in several pathways: glycolysis, glycogenesis, glycogenolysis gluconeogenesis and the pentose phosphate shunt. Thus the rate at which it is converted to glucose-1-phosphate is dependent

on the flux along these other pathways. However, the most important rate-limiting step in glycogenesis is glycogen synthetase. The activity of this enzyme is controlled by a cyclic adenosine 3',5'-monophosphate (c-AMP) dependent cascade. C-AMP dependent protein kinase phosphorylates glycogen synthetase I, giving rise to the phosphorylated inactive "D" form. This leads to the reduction of the rate of glycogen deposition.

Glycogenolysis is the process by which glycogen is broken down to glucose. The breaking of the α-1,4-glycosidic bond is catalysed by phosphorylase-a. The α-1,6 bond is broken by the debranching enzyme. The latter releases glucose while the former gives rise to glucose-1-phosphate which is converted to glucose-6-phosphate by phosphoglucomutase. Glucose is the product of the glucose-6-phosphatase reaction.

The key enzyme of glycogenolysis is phophorylase-a. Its activity is also dependent on the c-AMP cascade. C-AMP dependent protein kinase phosphorylates the inactive phosphorylase-b kinase to produce the active enzyme. The active phosphorylase-b kinase phosphorylates the inactive phosphorylase-b, converting it to the active "a" form. Phosphorylase phosphatase catalyses the reverse reaction, thus inactivating phosphorylase-a.

An important characteristic of the c-AMP cascade is that it activates phosphorylase-a and simultaneously inactivates

glycogen synthetase.

Glucose may also be synthetized in the liver from smaller molecules. This process is known as gluconeogenesis. Precursors such as lactate , pyruvate, glycerol and some amino acids can be converted to glucose. The pathway involves the same intermediates as glycolysis but different enzymes: pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose bisphosphatase. (For references on carbohydrate metabolism see Stryer, 1975 b,c,d; Mayer, 1979; Lehninger, 1975 a,b).

Though all non-reversible enzymes are potentially rate-limiting, it is believed that the flux through the so called "reverse glycolysis" is dependent on the relative activities of pyruvate kinase (conversion of PEP to pyruvate) and PEPCK. We will see below that pyruvate kinase activity can be rapidly modified, suggesting that it is the true rate-limiting step in the pathway.

It is important to realize that glycogenesis, glycogenolysis, gluconeogenesis and glycolysis operate simultaneously, and that whether the liver is taking up or releasing glucose depends on the relative magnitude of the fluxes through these pathways. In other words, if more carbon atoms go through glycolysis and glycogenesis than through glycogenolysis and gluconeogenesis, net glucose uptake will be observed.

These pathways are not only controlled by substrate availability, but also by hormonal and neural signals which change the activity of the rate-limiting enzymes. These are regulatory steps activated by homeostatic mechanisms and serve the glucose requirements of the organism. Insulin, glucagon, catecholamines, glucocorticoids and growth hormone (GH) are the major hormones resonsible for the regulation of carbohydrate metabolism.

Hormonal Control of Carbohydrate Metabolism

1) Insulin

Insulin is synthetized in the beta-cells of the islets of Langerhans. The first synthetic product is pre-proinsulin (Permutt, 1981) which is made up of 107 amino acids (in man). This is converted to proinsulin (84 amino acids) which is stored in the early secretory granules. Proinsulin is cleaved at two sites to give rise to insulin and C-peptide (Steiner and Oyer, 1967). Insulin is made up of the A and B chains, which are linked by two disulfide bonds, and which were initially connected by the 32 amino acids (in man) of the C-peptide. The vesicles, now containing insulin and C-peptide in equimolar amounts, are associated with microtubules and microfilaments. The latter have calcium dependent contractile properties, and these structures play an

important role in the process of secretion (Orci et al., 1973 and Orci et al., 1979). The fusion of the plasma membrane and the membrane of the secretory granule occurs as the latter is pulled to the plasma membrane. After fusion of the membrane of the vesicle with the plasma membrane, insulin and C-peptide are secreted in equimolar amounts by exocytosis.

Both the increase in the concentration of cytosolic calcium and c-AMP are linked to insulin release (Charles et al., 1975). Secretagogues cause a decrease in the plasma membrane permeability to potassium, resulting in the depolarization of the membrane. This in turn results in the opening of voltage dependent Ca^{2+} channels (Meissner, 1979). The increased Ca^{2+} concentration stimulates calmodulin activation of adenylate cyclase in the beta-cell (Valverde et al., 1979). Increased c-AMP concentration is believed to play an important role in the regulation of cellular calcium distribution (Hahm et al., 1980). Prolonged elevated levels of free cytosolic Ca^{2+} results in the release of insulin (Wollheim et al., 1975).

Insulin secretion is stimulated by metabolites such as glucose and some amino acids, hormones such as glucagon and gastric inhibitory peptide (GIP), and nervous stimuli.

The most potent secretagogue is glucose. Glucose stimulates both the secretion and synthesis of insulin. Two theories have been put forward to explain glucose-secretion coupling: 1) D-glucose interacts with a specific glucoreceptor which initiates events leading to secretion (McDaniel and Lacy, 1981). 2) Some intermediate of glucose metabolism is responsible for the mediation of insulin release. The latter theory is supported by the observation that non-metabolizable hexose analogues do not release insulin, whereas D-glyceraldehyde (glycolysis intermediate) not only does so, but also promotes insulin synthesis (Jain et al., 1975 and Ashcorft, 1981). The response of beta-cells to glucose is biphasic (Curry et al., 1968). The first phase is a sudden increase in the rate of secretion of insulin, which peaks in minutes. The rate reaches its nadir about 20 min later. The second phase is a sustained increase in the secretion rate, which lasts as long as ambient glucose concentration is elevated. Amino acids such as alanine and leucine promote insulin release but not its synthesis. Other amino acids have little or no effect (Ashcroft, 1981).

Glucagon stimulates insulin release independently of its hyperglycemic effect (Samols et al., 1965). GIP at or above plasma levels observed postprandially stimulates insulin secretion in the presence of glucose (Dupre et

al., 1973). Somatostatin inhibits insulin secretion (Alberti et al., 1973).

Stimulation of the parasympathetic nervous system leads to insulin secretion. Cholinergic agents act similarly. The stimulation of the sympathetic nervous system results in the inhibition of insulin release. However β-adrenergic stimuli facilitate insulin secretion, whereas α-adrenergic stimuli are inhibitory (Wood and Porte, 1974).

In adipocytes and muscle cells insulin is known to increase the permeability of the cell to hexoses, amino acids and ions such as K^+ and PO_4^{3-} (Czech, 1980 and Hepp et al., 1968). In the liver insulin inhibits both glycogenolysis and gluconeogenesis (Soling and Senfert, 1975). In both liver and muscle it stimulates glycogen deposition (Cherington et al., 1982 and Shulman et al., 1978). The hormone promotes protein synthesis in muscle and some other tissues (Wool, 1975). It inhibits triglyceride hydrolysis and stimulates fatty acid synthesis and fat deposition in adipocytes (Jungas, 1975).

The biological effects of insulin are dependent on the binding of the molecule to a specific receptor on the surface of the target cell. The maximal effect occurs when approximately 10% of the receptors are occupied.

Thus 90% of the total available receptors are "spare receptors" (Olefsky, 1981). The receptor is a glycoprotein believed to be a tetramer made up of two large α and two smaller β subunits arranged symmetrically and connected by disulfide bonds with the following stoichiometry: (α-s-s-β)-s-s-(α-s-s-β) (Massague et al., 1980 and Czech, 1981).

The interaction of insulin and its receptor results in the release, by proteolysis, of a small peptide of membrane origin (Seals and Czech, 1980). This peptide, or membrane factor, activates pyruvate dehydrogenase as well as inhibits c-AMP binding to protein kinase resulting in the observed decrease of protein kinase activity (Seals and Czech, 1980 and Walkenback et al., 1978). The same factor also activates glycogen synthetase phosphatase (Larner et al., 1979), thus increases the rate of glycogenesis. It is assumed that the membrane factor is not a degradation product of insulin, since the binding of other molecules such as concanavalin A or antibodies to the receptor also cause the release of the peptide (Czech, 1981).

The binding of insulin to its receptor also changes the permeability of the cell membrane to metabolites. This effect, probably independent of the release of the membrane factor, is due to a change in the local

viscosity of the membrane. Thus local membrane protein redistribution due to insulin binding may result in a localized increase in the fluidity of the membrane leading to increased permeability (Czech, 1980).

The long term effects of insulin involves the control of gene expression by an, as of yet, unknown mechanism. Hepatocytes exposed to insulin for 24 h contain increased amounts of glucokinase. This increase does not occur in the presence of either transcription or translation inhibitors (Katz et al., 1979). Direct quantitative measurement of mRNA indicates that the decrease in synthesis of PEPCK, observed when hepatocytes are exposed to varying concentrations of insulin, coincides with a decrease in the amount of mRNA coding for the enzyme (Granner et al., 1983).

2) Glucagon

Glucagon is a protein synthetized in the alpha-cells of the islets of Langerhans. It consists of 29 amino acids, and has a molecular weight of 3,500 daltons (Orci et al., 1983). It is synthetized as a large molecular weight precursor, pre-proglucagon, with a size of 18,000 to 19,000 daltons. This molecule subsequently undergoes several cleavages which lead to glucagon. Pulse chase studies have revealed that pre-proglucagon gives rise to

smaller glucagon immunoreactive proteins with molecular size of 19, 18, 13, 4.5, 3.5 kilodaltons (Noe et al., 1981). One of the fragments, made up of 100 amino acids with a molecular weight of 12,000 daltons, has been identified as proglucagon and named glicentin (Unger and Orci, 1981). This process occurs in the secretory granules. Glucagon is secreted by exocytosis, involving a mechanism similar to that of insulin release (Carpantier et al., 1977).

Vranic et al. (1974) found, in pancreatectomized fasting dogs, plasma concentration of IRG similar to that observed in normal dogs. Fasting for 5 to 6-7 days resulted in an increase in IRG secretion so that plasma concentration became 6 to 9 times normal. They concluded that glucagon is secreted by other tissues than the pancreas, and proposed the gastric mucosa as the site of extrapancreatic glucagon release. A quarter of the total amount of glucagon is released by the gastrosplanchnic area in dogs (Muller et al., 1978). In humans extrapancreatic glucagon is virtually non-existent (Bloom et al., 1976).

Glicentin is synthetized in the cells of the intestinal mucosa, as well as in the alpha-cells. However the former do not have the necessary enzymes to process proglucagon to glucagon. Circulating glicentin does not

have the full biological activity of glucagon but contributes to glucagon-like immunoreactivity (GLI) measured in plasma.

The most important stimulus for glucagon secretion is hypoglycemia. The lower the glucose concentration the more glucagon is released (Itoh et al., 1981). Gut hormones such as GIP, vasoactive intestinal petide (VIP), gastrin and pancreozymin (Itoh et al., 1981 and Montague, 1983) all stimulate the secretion of glucagon. Sympathetic and parasympathetic stimulation enhance glucagon secretion (Itoh et al., 1981 and Woods and Porte, 1974). Glucagon release is also stimulated by epinephrine (Gerich et al., 1976). Glucagon secretion is inhibited by glucose at physiological insulin concentration (Unger et al., 1972), by somatostatin (Koerber et al., 1973) and by insulin at high glucagon levels (Samols et al., 1969).

In vivo experiments on conscious dogs have shown that exogenous (Cherrington et al., 1981) or endogenous (Gauthier and Hetenyi, 1982) glucagon stimulates glycogenolysis and gluconeogenesis. Studies using perfused rat livers from fed or fasted animals demonstrated that glucagon and c-AMP increase glucose production, decrease the amount of glycogen stored in the liver and increase glucose synthesis to the same extent

(Exton and Park, 1969). These observations support the theory that the effect of glucagon is mediated by the second messenger c-AMP.

The biological effect of glucagon is dependent on the binding of the hormone to its specific membrane receptor. The molecular structure of the receptor is unknown. However it has been postulated that it consists of a receptor region and a GTP binding region. When glucagon binds to the receptor region, GTP activates the receptor complex which in turn activates adenylate cyclase, which is also a membrane protein (Rodbell, 1980).

The rapid effect of c-AMP on the regulation of glycogenesis and glycogenolysis has been discussed above. The rapid effect of glucagon on gluconeogenesis involves the inactivation of pyruvate kinase (PK) by phosphorylation, via c-AMP dependent protein kinase (Riou et al., 1976 and Riou et al., 1978). The inactivation of PK favors a net gluconeogenic flux at the level of the pyruvate substrate cycle. Glucagon and c-AMP cause a rapid decrease in the activity of PFK and a rapid increase in the activity of fructose bisphosphatase in rat liver (Taunton et al., 1972). This also favours a net gluconeogenic flux at the level of the fructose 6-phosphate:fructose 1,6-bisphosphate substrate cycle. The

activity of fructose bisphosphatase is known to increase when the enzyme is phosphorylated, however it is not known if c-AMP dependent protein kinase is involved in the phosphorylation (Pilkis et al., 1978). PFK, is also activated by phosphorylation. Thus a cascade mechanism which would involve the phosphorylation and consequent activation of a phosphatase, with PFK as substrate, is postulated (Pilkis et al., 1978).

A slow effect of glucagon and c-AMP is to increase the activity of PEPCK (Pilkis et al., 1978). Glucagon or c-AMP injections into livers of fetal rat _in utero_ result in the synthesis of PEPCK and mRNA coding for PEPCK prior to parturition (Hanson et al., 1973 and Ruiz et al., 1978). Similar observations were made in hepatocytes from adult rats (Iynedjian and Hanson, 1977). Glucagon also accelerates the translation of pre-existing mRNA for the enzyme (Schut, 1980).

3) Catecholamines

The catecholamines epinephrine, norepinephrine and dopamine are amino derivatives of 1,2-dihydrobenzene (Grodsky, 1979). Their synthesis begins with the transformation of thyrosine to dihydroxyphenylalanine (DOPA), which is converted to dopamine which gives rise to norepinephrine and finally epinephrine (Stryer,

1975a). This process occurs in the adrenal medulla and in the adrenergic neurons. Epinephrine and to a lesser extent norepinephrine are secreted into the circulation by the adrenal gland in response to sympathetic stimulation. Norepinephrine and dopamine are neurotransmitters which are released by the presynaptic terminals of adrenergic neurons (Ganong, 1979). Some "leak" into the circulation, raises the norepinephrine/epinephrine ratio in systemic blood above that found in the adrenal effluent blood. Catecholamines are released in response to stresses such as hypoglycemia, hypoxia, hypotension, emotional stress and heavy excercise.

An elevated plasma epinephrine concentration results in an increased rate of production, and a decreased rate of utilization of glucose (Gerich et al., 1980), among the other well known metabolic and cardiovascular effects. Studies on perfused rat livers from fed or fasted animals show that epinephrine increases glucose production, decreases the total amount of glycogen and increases glucose synthesis (Exton and Park, 1969). It also prevents insulin induced glucose uptake by muscle (Sloan, et al. 1978). In the intact animal it decreases the rate of metabolic clearance of glucose. Elevated epinephrine concentration above 400 $pg.ml^{-1}$ are reported

to decrease plasma concentration of insulin. (Clutter et al., 1980 and Rizza et al., 1979).

The effects of catecholamines are initiated by their binding to one of two classes of receptors known as α and β receptors. These are responsible for the α- and β-adrenergic responses. The β-receptor is associated with GTP binding and activation of adenylate cyclase (Orly and Schramm, 1976). The activation of gluconeogenesis and glycogenolysis by c-AMP has been discussed above. Gluconeogenesis is also stimulated by a α-adrenergic mechanism. A-adrenergic agonists activate glucose synthesis while c-AMP concentration is low (Pilkis et al., 1978). A cellular redistribution of Ca^{2+} is believed to be involved in this mechanism (Assimacopoulos-Jeannet et al., 1977). How this would stimulate glucose synthesis is unknown.

4) Glucocorticoids

Glucocorticoids are C21 (21 carbons) steroids. They are synthetized in the adrenal cortex from their precursor cholesterol. The three most potent glucocorticoids are cortisol, cortisone and corticosterone. Their secretion is under the control of adrenocorticotropic hormone (ACTH), secreted by the anterior pituitary. The release of ACTH is regulated by

corticotropic releasing hormone (CRH) which is synthetized in the hypothalamus. ACTH interacts with membrane receptors of the adrenal cortex cell, and activates adenylate cyclase. The increase in c-AMP results in the secretion of adrenal corticoids. Glucocorticoids suppress the release of both ACTH and CRH (Jensen, 1980).

Glucocorticoids have both catabolic and anabolic effects. The catabolic effects relate to peripheral tissues, such as muscle, lymphoid tissue, connective tissue, and adipose tissue. In all these, protein degradation is increased in the presence of elevated levels of glucocorticoids. These steroids will also increase lipolysis and cause the release of fatty acids and glycerol from adipocytes (Baxter and Farsham, 1972). Therefore more gluconeogenic substrate becomes available to the liver, thus in this way steroids promote hepatic glucose output (Hetenyi et al., 1980b).

Glucocorticoids do not have a direct regulatory role in short term glucoregulation but by their "permissive" effect on other hormones they modulate it. Continuous glucocorticoid infusion has either no effect on the glucose concentration and the rate of glucose production or slightly decreases both (Eigler et al., 1979 and Ninomiya et al., 1965). If physiological infusions of

glucagon or epinephrine are given simultaneously with cortisol, the response to these hormones is significantly augmented (Eigler et al., 1979).

Long term exposure to elevated glucocorticoid concentrations increases the turnover rate of glucose in dogs as well as increases plasma insulin, while plasma glucose concentration remains within the normal range (Ninomiya et al., 1965 and Campbell and Rastogi, 1976).

Steroid hormones diffuse through cell membranes, and interact with cytosolic protein receptors to form receptor-hormone complexes. The receptors have high specificity and high affinity for their hormone. The complex, translocated to the nucleus, reacts with non-histone proteins resulting in the initiation of transcription of specific segments of DNA (O'Malley, 1976). The synthesis of gluconeogenic and glycogenolytic enzymes, stimulated by glucocorticoids, is believed to be the basis of the permissive action of these hormones (Schut, 1980; Chan et al., 1979 and Iynedjian et al., 1978).

5) Growth hormone (GH)

Growth hormone is a single chain protein secreted by the somatotropes of the anterior pituitary. Its release is under the control of the hypothalamic somatotropin

releasing factor and the somatotropin releasing inhibitory factor (SRIF), also known as somatostatin (Jensen, 1980). Growth hormone in large doses (1 mg) has transient insulin-like effects (Merimee and Rakin, 1973), but anti-insulin effects are observed when GH is chronically ($1 mg.kg^{-1}.day^{-1}$) administered to dogs. However glycogen content of the liver is reported to be twice that of control (Biship et al., 1967). Insulin concentration in the plasma rises but the change in the rate of glucose utilization is much less than what would be expected, thus suggesting peripheral insulin resistance (Hetenyi et al., 1983).

The infusion of glucose to normal humans, started 4 h after a physiological infusion of GH was started, caused a larger increase in plasma glucose than glucose given without prior GH infusion. The degree of the suppression of hepatic glucose production by glucose was less than in the control dogs, and the increase in metabolic clearance was less (MacGorman and Rizza, 1980). These observations suggest that at physiological concentration GH may play a permissive role in glucoregulation.

Hepatic Glucose Autoregulation

Autoregulation is the ability of the liver to modify its

glucose kinetics, in the absence of any hormonal changes, in response to changes in ambient glucose concentration.

Studies on perfused rat livers indicate that perfusion with a solution containing no glucose brings about a net glucose release and glycogenolysis, while perfusion with a solution containing 335 mg.dl^{-1} of glucose causes a rapid net glucose uptake and glycogen synthesis which are proportional to the glucose concentration in the perfusate (Glinsmam et al., 1969). Similarly, when the perfused dog liver is exposed to increasing glucose concentration a net release of glucose is converted to a net uptake (Bucolo et al., 1974).

Studies by Davidson et al. (1981), Liljenquist et al., (1979) and Shulmam et al., (1979) show that in the absence of insulin hyperglycemia does not lead to glycogen synthesis even though net hepatic glucose uptake is observed. It is possible that insulin maintains the activity of the rate-limiting enzymes glycogen synthetase and glucokinase, such that substrate availability would control the flux to glycogen.

Glucose may act as an allosteric effector. Long term exposure of rat hepatocytes to elevated glucose concentrations enhances glucokinase synthesis (Katz et al., 1979). Glucose is known to increase the activity of phosphorylase phosphatase. (Hers et al., 1970). This latter increase is associated with glucose binding to phosphorylase-a which then becomes more susceptible to the action of the phosphatase (Chan et al.,

1979). This decreases the rate of glycogen breakdown.

Normal Glucoregulatory Responses to Hypoglycemia

In normal healthy individuals plasma glucose concentration is kept constant. In the post-absorptive state (i.e. when no absorption of nutrient from the intestinal tract takes place) it is about 95 to 115 $mg.dl^{-1}$ in dogs and 80 to 100 $mg.dl^{-1}$ in humans. When events cause the level of glucose to rise or fall the organism puts into action mechanisms by which glycemia is returned to its basal level. Glucose counterregulation occurs when the net effect of these mechanisms counters the fall of plasma glucose concentration.

In dog, when plasma glucose concentration is lowered by 15 - 20%, the rate of glucose production (Ra) is increased so as to return the level of glucose closer to normal (Gauthier et al., 1980) (See appendix A for a summary of these experiments). The rise in Ra was also demonstrated to be due to an increased release of glucagon into the plasma. The authors concluded that increased glucagon secretion is the response to a moderate decrease of plasma glucose since the increase in Ra can be prevented when the release of glucagon is inhibited by somatostatin. The same investigators also reported that during overt hypoglycemia (glucose concentration below 65 $mg.dl^{-1}$), produced by an infusion of insulin, the increase in glucose production is not abolished by

somatostatin and is believed to be mediated by catecholamines. Direct measurments revealed, in human subjects, that the concentration of epinephrine and norepinephrine do rise during insulin induced hypoglycemia (Gerich et al., 1981). Recovery from hypoglycemia is slowed down when the increase in glucagon secretion is abolished by somatostatin. However, a larger increase in epinephrine secretion causes Ra to rise. When the effect of catecholamines is blocked by phentolamine and propanolol, the recovery from insulin induced hypoglycemia is at a normal rate. When somatostatin and the adrenergic blockade are used simultaneously, recovery does not occur (Rizza et al., 1979). When the same adrenergic blockade is used during moderate hypoglycemia, the recovery is normal. It is delayed, however, when glucagon release is also inhibited (Cryer et al., 1981). This is consistent with the report that the concentration of catecholamines remains constant while that of glucagon increases in dogs exposed to a similar degree of hypoglycemia (Gauthier et al., 1983).

In overt hypoglycemia, increases in the plasma concentration of growth hormone (GH) and cortisol have been reported. However, the inhibition of GH secretion by somatostatin (with glucagon replacement) or the suppression of glucocorticoid secretion by adrenalectomy, do not delay the return of plasma glucose to the basal level (Gerich et al., 1980). Moderate hypoglycemia does not change the plasma

cortisol concentration (Gauthier et al., 1983).

Based on these observations it was proposed (Gauthier et al., 1980) that the effect of glucagon is responsible for non-hypoglycemic glucose counterregulation, whereas both glucagon and catecholamines are involved in the recovery from overt hypoglycemia. Gauthier and Hetenyi (1982) demonstrated that the hepatic effects of glucagon released in response to a moderate hypoglycemic stimulus is a rapid increase in glycogenolysis followed by a slower increase in gluconeogenesis resulting in a sustained increase in Ra. This report was consistent with the observation by Cherrington et al. (1981), who found that a 4 fold increase in the level of glucagon leads to increased rates of gluconeogenesis and glycogenolysis resulting in a 3 fold increase in Ra.

An elegant study by Santiago et al. (1980) demonstrated that the stimulus for counterregulation is a rapid drop in plasma glucose. They found that when plasma glucose concentration is decreased from 200 to 100 $mg.dl^{-1}$ or from 95 to 65 $mg.dl^{-1}$, an increase in the concentration of glucagon, epinephrine and norepinephrine is observed. The magnitude of the response is greater when the fall is from 95 to 65 $mg.dl^{-1}$. The authors therefore postulated that the magnitude of the response is related to the absolute glucose concentration but that the rate of decrease is also a contributing factor in eliciting a response, since Sacca et al. (1979) had reported

that a slow change from 90 to 67 $mg.dl^{-1}$ does not bring about counterregulation.

III Chemically Induced Diabetes

The discovery of Dunn et al. (1943) that alloxan produces diabetes mellitus in rabbits, promoted the use of cytotoxic agents, more or less specific for beta-cells, for the experimental induction of diabetes.

Effect of Alloxan on Plasma Glucose Concentration

A single intravenous injection of a diabetogenic dose of alloxan produces a triphasic change in plasma glucose. Between 1 and 4 h after the injection an initial hyperglycemia is observed. This is followed by a period of transient hypoglycemia 6 to 12 h after the injection, which if too severe may cause death (Rerup, 1970). A permanent hyperglycemia follows, some 12 to 24 h postinjection (Cooperstein and Watkins, 1981).

The first phase may be of pancreatic origin. A modest increase of pancreatic insulin content has been reported, suggesting that the secretion of insulin is initially inhibited (Dixit et al., 1962). Nevertheless a hepatic effect is certain to contribute since the injection of alloxan increases the release of epinephrine, which stimulates hepatic glucose production. Indeed adrenalectomy abolishes or reduces

the initial hyperglycemia (Boquist and Lorentzon, 1980).

The deep hypoglycemia in the second phase is due to an increase in plasma concentration of insulin (Lundquist and Rerup, 1967), due to its release from destroyed beta-cells. There is good evidence that the fall in glucose concentration is not due to the direct effect of alloxan on the liver (Kennedy and Lukens, 1944).

The permanent hyperglycemia is clearly the result of insulinopenia.

Effect of Alloxan on the Beta-cell

Light microscopy has revealed a time sequence for the destruction of beta-cells of the islets of Langerhans by alloxan. This sequence is identical in the rabbit, rat, dog as well as the toadfish (Cooperstein and Watkins, 1981). Five minutes after the exposure to alloxan a decrease in the number of nuclear and cytoplasmic granules is observed (Bailey et al., 1944). After 10 to 60 min following alloxan treatment the cytoplasm becomes vacuolated, the nuclei are pycnotic and the cells have shrunk (Lazarus et al., 1962). After 3 h the beta-cells are detached from one another. Nuclear karyolysis and disintegration of the nuclear membrane is observed after 5 h (Cooperstein and Watkins, 1981). Within 24 h the islets are made up mostly of alpha-cells (Lukens, 1948). Electron microscopy reveales that alloxan treatment causes the

destruction of membrane bound organelles such as the endoplasmic reticulum and mitochondria. The plasma membrane is disrupted as well. (Cooperstein and Watkins, 1981; Orci et al., 1972; Orci et al., 1976 and Grankvist et al., 1972). The destruction of the beta-cell is permanent and non-reversible.

Effects of Alloxan on Tissues Other than Beta-cells

1) Alpha-cells

Light microscopy studies show that alpha-cells survive diabetogenic or even lethal doses of alloxan, whereas beta-cells are destroyed. Though the alpha-cells are alive, their morphology is somewhat altered: they are swollen and rounded showing increased granulation (Dunn et al., 1944).

2) Kidney

Since the discovery of the diabetogenic effect of alloxan was a result of "crush kidney" syndrome research (McLetchie, 1982) it is obvious that alloxan does cause kidney lesions. The glomeruli are usually normal whereas the luminal side of the convoluted tubules are often the site of vacuolization, necrosis and desquamation. These lesions are most conspicuous during the first four days after the alloxan injection, after which time they tend to disappear. In late diabetes the kidneys are usually normal. In rats these lesions occur when the dose is greater than

40 mg.kg^{-1} (Lukens, 1948).

3) Liver

In dogs a very large dose of alloxan will cause hepatic lesions such as central lobular necrosis, and fatty infiltration. In some cases jaundice has been reported. Low diabetogenic doses, in the rat, produce focal necrosis of peripheral lobules (Lukens et al., 1948). One must be cautious in interpreting these findings, since diabetes in itself will cause hepatic changes such as fatty infiltration. Thus it is not clear which of these lesions are the result of alloxan and which of diabetes.

IV Phlorizin and the Study of Counterregulation

To separate the effects of moderate hypoglycemia per se from those of hyperinsulinemia, we have used phlorizin in the experiments to be reported. Phlorizin, a polyhydroxyphenol glycoside, has a high affinity for D-glucose receptors, believed to be part of the glucose transport system of the brush border of the proximal kidney tubule (Silverman and Black, 1975). When infused intravenously to dog (6 μg. kg^{-1}.min^{-1}) D-glucose reabsorption at the luminal surface is completely blocked (Silverman et al., 1970). This results in persistent glycosuria (Nash, 1927).

A thousand times more phlorizin is necessary to inhibit the

canine hepatic D-glucose transport system (Goresky and Nadeau, 1974). For these reasons the glycoside has been used to increase the rate of glucose removal from the circulation, in the post-absorptive (Gauthier et al., 1980) and 4 day fasted dog (Gauthier and Hetenyi, 1982), without directly interfering with the release of glucagon or hepatic glucose production. In dogs, during the infusion of phlorizin, the concentration of glucose in the plasma is reduced by approximately 15%. This brings about an increase in the rate of glucose production mediated mainly by the release of glucagon (Kolodny et al., 1962 , Gauthier et al., 1980, Gauthier and Hetenyi, 1982 , Gauthier et al., 1983) (See table 6, appendix A).

V The Aim of this Project:

The aim of this project was to study the glucoregulatory response to hypoglycemia in diabetic dogs. Special efforts were taken to differentiate between the response to moderate hypoglycemia without hyperinsulinemia and hypoglycemia with hyperinsulinemia. Six question were asked:

1) Is there an increase in the rate of hepatic glucose production when plasma glucose is decreased by an infusion of phlorizin?
2) Does such an increase occur when plasma glucose is decreased by an infusion of insulin?
3) Is the plasma concentrations of glucagon and/or catecholamines increased during either infusion?

Since diabetes, in dogs, can be characterised by a low plasma insulin concentration, a high plasma glucagon concentration and a high rate of hepatic glucose production as well as an elevated plasma glucose concentration, we asked the next three questions:

4) Can a one week long insulin treatment, which maintains normoglycemia in the post-absorptive state, restore counterrgulation in alloxan-diabetic dogs subjected to a phlorizin infusion?

5) If plasma glucagon concentration of diabetic dogs were kept at a near normal (non-diabetic) level, as it is in pancreatectomized dogs, would there be an increase in hepatic glucose release in moderate and overt hypoglycemia?

6) Do high rates of gluconeogenesis and hepatic glucose production, without the low diabetic IRI/IRG ratio prevent counterregulation during moderate or overt hypoglycemia in dogs?

CHAPTER II

MATERIALS AND METHODS

I The Animals

Normal healthy mongrel dogs, weighing from 6.1 kg to 18.9 kg, and unselected for sex were used for these experiments. The animals were vaccinated against distemper, adenovirus (type I and II), leptospirosis, parainfluenza, parvovirus and rabies. All the dogs were monitored for intestinal parasites and treated accordingly. They had access to food and water *ad libitum*. The diet contained 49% protein, 8.4% fat, 32% carbohydrate and 10.5% fiber on a dry weight basis. Alloxan-diabetic dogs were fed, at specific times of the day, a palatable meal of canned dog food containing 75% moisture and 40% protein, 16% fat and 38% carbohydrate on a dry weight basis, which gave a caloric content 1.139 kcal.g^{-1} wet weight. These dogs had access to water *ad libitum*.

A - Chemical Induction of Diabetes

1) Induction

Intitially a mixture of streptozotocin and alloxan at sub-diabetogenic doses (Issekutz, 1974) (30 mg.kg^{-1} streptozotocin and 50 mg.kg^{-1} alloxan) was used. However,

of the three dogs treated in this fashion one needed a second injection, one died in deep hypoglycemia, and only one developed diabetes after the first injection. All three were violently sick within the first 24 hours. We finally opted for an intravenous injection of a 13% solution of alloxan monohydrate in a 0.1 M acetate buffer at pH 4.4 at a dose of 65 $mg.kg^{-1}$ (0.5 $ml.kg^{-1}$). Out of the ten dogs injected, eight became diabetic.

The dogs were fasted 18 to 20 hours before the injection. A polyethylene catheter (Clay Adams PE 190) was inserted, under local lidocaine HCl (10 $mg.ml^{-1}$) anaesthesia, into the vena cava via a saphenous vein. The animal was allowed to rest in a Pavlov stand for 50 minutes before the injection.

2)Maintenance

To avoid any untoward effects of the transient hypoglycemia expected 6 - 12 hours after the injection of alloxan, the dogs were fed three times within the first 24 hours following the injection. After this initial period the animals were fed twice daily with an initial maintenance caloric intake of 110 $kcal.kg^{-1}$ per day. If the animal lost weight, the amount of food was increased. One third of the animal's ration was given at 8:30 h, half an hour before the insulin injection. The remaining

2/3 were given between 12:00 and 13:00 h.

All diabetic dogs were housed in metabolic cages suitable for urine collection. Three daily samples, the first nominally an overnight sample, the second a morning sample and the third an afternoon (17:00 h) sample, were monitored for glucose. Urine glucose concentration was estimated using Clinitest tablets (Ames). Blood samples were drawn ever two days, in the morning (18 - 12 hours post-absorptive), and plasma glucose concentration was measured with a Beckman Glucose Analyser 2. When glycosuria greater than 150 $g.dl^{-1}$ and glycemia greater than 250 $mg.dl^{-1}$ were observed, insulin therapy was started. The initial dose was 1 $U.kg^{-1}$ per day given subcutaneously as a mixed injection of crystalline zinc insulin and protamine zinc insulin (PZI). The dose was subsequently adjusted according to glycosuria and glycemia by daily increment of 2 U. Once the animal was stabilized it was maintained on PZI only. The insulin was usually administered 30 minutes after the morning meal so that glucose absorption was not completed when the effect of the fast acting insulin peaked, approximately 3 hours after injection. If the afternoon urine sample did not have a glucose concentration lower than the morning sample, the dog was given a supplementary 5 to 10 U of crystalline zinc insulin.

B - Pancreatectomy

1) Surgical procedure

A total pancreatectomy was performed, as described by Markowitz (1959) on 4 healthy dogs. Special care was taken to use nonabsorbable sutures (stainless steel) to close the peritoneum.

2) Maintenance

The resulting pancreatic insufficiency was treated by adding dry porcine pancreatic enzyme concentrate to the normal food.

Urine glucose concentration was monitored every morning. Plasma glucose was measured every two days. Insulin was injected as a mixture of crystalline zinc insulin and PZI, starting 22 hours postoperatively and every 24 hours thereafter. The initial dose of 1 $U.kg^{-1}$ per day was increased by an increment of 2 U daily until diabetes was under control. The full dose was then injected as PZI alone.

C - Methylprednisolone Treatment

Normal nondiabetic dogs were treated with 3 $mg.kg^{-1}$ methylprednisolone acetate (Depo-Medrol) i.m. for 4 consecutive days. On the 5th day the first experiment was carried out. A fifth methylprednisolone injection was given on

the 6th day and the last experiment was carried out on the following (i.e. 7th) day.

D - Antibiotics

Following any procedure which involved a skin incision, 100,000 IU penicillin G benzathine, 100,000 IU penicillin procain and 250 mg of dihydrostreptomycin per 10 kg body weight (10 ml Penlong S - Rogar/STB, Montreal, Que., Canada) were administered i.m.. A second identical injection was given two days later. If signs of infection (fever, festering of wounds) were observed supplementary injections were given, and wounds were treated with topical antibiotics (NeoSporin, Calmic Medical, Kirkland, Ont., Canada).

II Procedures

A.- Cannulation

All dogs used were in the post-absorptive state following an 18 to 20 hour fast. On the day of the experiment, two indwelling polyethylene catheters were inserted into the cephalic and saphenous veins under local lidocain anaesthesia. The cannulations were performed using a cut-down ligation techinque. The cephalic catheter was a concentric double barrelled cannula (PE 60 in a PE 205) for the simultaneous unmixed infusion of [3-^{3}H]-glucose and phlorizin or insulin.

The tip of the saphenous cannula was pushed up to the inferior vena cava and used for the injection of the [3-^{3}H]-glucose primer and for blood sampling.

At the end of each experiment the saphenous cannula was removed and the vein was ligated. The wound was closed and dressed with Nitrofurasone Soluble Dressing (Armitage and Carrol, London, Ont., Canada). If more than two infusions were to be carried out on a dog, the "nick" in the cephalic vein was sutured with 6-0 vascular silk and blood flow was reestablished. Otherwise the vessel was ligated.

B - Blood Samples

Blood samples of 2 to 6 ml were drawn from the vena cava, and immediately transferred to pre-cooled heparinized plastic tubes and kept on ice. The sampling cannula was flushed with an equivalent volume of 0.9% saline after each sampling.

C - Preparation of Blood Samples for Hormone and Metabolite Determinations

Blood samples used for the determination of glucose concentration and specific activity (SA), were centrifuged at 2,000 rpm (revolutions per minute) (1,000 x g) for 10 min at 4° C no later than 30 min after withdrawal. Samples for the determination of free fatty acids (FFA) concentration were spun at 2,000 rpm (1,000 x g) for 10 min at 4° C, 5 min after

they were drawn.

Blood samples of 3.25 ml were drawn for glucagon and insulin determinations. The blood was immediately transferred to pre-cooled tubes, containing disodium ethylenediaminetetracetate (EDTA - 25 $mg.ml^{-1}$) - 1.2 $mg.ml^{-1}$ blood - as an anticoagulant, and the protease inhibitor aprotanin (50,300 $KIU.ml^{-1}$) - 770 $KIU.ml^{-1}$ blood. A micro-hematocrit was done on the diluted blood so as to calculate a correction factor for the assays. The blood was centrifuged at 2,000 rpm (1,000 x g) for 10 min. The plasma was separated and two 0.8 ml aliquots were frozen at -20° C and later transferred to -70° C.

One milliliter of blood for catecholamine determination was transferred to a polypropylene tube containing 2.5 mg of glutathione and 0.01 ml of ethyleneglycol-bis-(β-aminoethyl ether) N,N,N',N'-tetra-acetic acid (EGTA - 100 $mg.ml^{-1}$) was added as an anticoagulant and kept on ice. The blood was centrifuged, no later than 30 min after withdrawal, at 2,500 rpm (1,800 x g) for 30 min at 4° C. An aliquot of 0.45 ml plasma was deproteinized with 0.05 ml of 2 N perchloric acid (PCA - $HClO_4$) on ice and centrifuged for 30 min at 16,000 rpm (30,000 x g) at 4° C. The clear supernatant was decanted and frozen at -20° C and later transferred to -70° C.

Catecholamine, glucagon and insulin samples were shipped to Toronto packed in dry ice.

III Experimental Design

Three series of experiments were carried out. The first two dealing with glucoregulation in diabetic dogs, the third with glucoregulation in dogs with elevated rates of gluconeogenesis and hepatic glucose production.

A - The First Series - Alloxan-diabetic Dogs

On four animals three types of experiments were carried out in radom order. On two other dogs, only Type I experiment and in another, Type III was performed, and one additional animal was subjected to two experiments (Type I and II). Thus altogether 7 experiments of Type I, 5 of Type II, and 5 of Type III were carried out.

Before the injection of alloxan, 3 blood sample were drawn ever 20 min over a 40 min period under the same physical conditions as during the experiments. These samples were processed as described above for insulin and glucagon determinations. Experiments were carried out at least 8 days after the alloxan injection. The dogs were given one week to recover between experiments.

1) Type I experiments

The dogs were in the diabetic state (72 hours after the last insulin injection), with a plasma glucose concentration betweer 250 and 450 mg.dl^{-1}. Following the

priming injection of 25 μCi of [3-^{3}H]-glucose, an infusion of saline (0.025 ml.min^{-1}.kg^{-1}) was given for 100 min with a Sage syringe pump model 335 (Sage Instruments Inc., White Plains, N.Y.). This was followed over the next 200 minutes (i.e. until t = 300 min) by the infusion of 0.05 mg.kg^{-1}.min^{-1} phlorizin at the same flow rate. During the whole length of the experiment a 0.25 μCi.min^{-1} [3-^{3}H]-glucose infusion (0.155 ml.min^{-1}) was maintained with a Harvard Compact Infusor model 975 (Harvard Apparatus Company Inc., South Natik, MA).

2) Type II experiments

The dogs were in the "treated-diabetic" state (24 hours after the last injection of insulin). The plasma glucose concentration was between 65 and 105 mg.dl^{-1} at the time of the experiment. The sequence and duration of the infusions were the same as during Type I experiments.

3) Type III experiments

The animals were in the diabetic state. The initial 100 min infusion of saline was changed to an infusion of 7 mU.kg^{-1}.min^{-1} insulin for 95 min. At t = 195 min, this was changed again to saline for 65 min (i.e. until t = 260 min).

B - The Second Series - Pancreatectomized Dogs

Type I and Type III experiments were carried out on four pancreatectomized dogs in random order.

C - The Third Series - Methylprednisolone Treated Dogs

Four methylprednisolone treated dogs were used, and each was subject to 2 experiments. A Type III design was used for both a phlorizin and an insulin infusion using the above mentioned dose of 0.05 $mg.kg^{-1}.min^{-1}$ and 7 $mU.kg^{-1}.min^{-1}$, respectively.

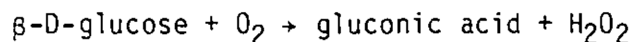
IV Blood Sampling Schedule

In the experiments of Type I and Type II design, 20 samples were drawn at t = 15, 30, 50, 65, 80, 90, 100, 110, 120, 135, 150, 165, 180, 195, 210, 225, 240, 260, 280 and 300 min. In experiments of Type III design, 18 samples were taken on an identical schedule but ending with the sample taken at t = 260 min. Glucose and radio-active glucose were determined in all samples. Free fatty acids, insulin, glucagon and catecholamines were determined at t = 65, 90, 100, 120, 150, 180, 210 and 260 min and also at t = 300 min in experiments of Type I and II designs. Catecholamines were measured for Type I and III experiments of the first series only (last 4 dogs). FFA determinations were not carried out in the second series.

V Determination of the Concentration of Metabolites and Hormones in the Plasma

A - The Determination of the Concentration of Plasma Glucose ($mg.dl^{-1}$)

The concentration of plasma glucose was determined from a 0.01 ml plasma sample with the help of a Beckman Glucose Analyser 2 (Beckman Instruments Inc., Fullerton, CA). The principle underlying the determination is as follows: the enzyme glucose oxidase (EC 1.1.3.4), catalyses the breakdown of β-D-glucose to gluconic acid and peroxide in the presence of oxygen.



$$\beta\text{-D-glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2$$

The maximum rate of oxygen consumption, as monitored by an oxygen electrode is directly proportional to the amount of glucose in a sample of fixed volume (Kardish et al., 1968).

B - The Determination of the Concentration of [3-^{3}H]-glucose in the Plasma (C*, $dpm.ml^{-1}$)

The concentration of labelled glucose in each sample was expressed as disintegration per minute per milliliter ($dpm.ml^{-1}$) of the isotope ^3H which releases the high energy electron of β-radiation.

The third carbon of glucose gives rise to the first carbon

of lactate which is a carboxyl carbon (Stryer, 1975c), thus [3-^{3}H]-glucose does not give rise to tritiated three carbon metabolites. It is, therefore, not necessary to isolate glucose chemically so as to determine its radio-activity. However, tritiated water is present in the samples. To remove tritiated water, the plasma is deproteinized with $Ba(OH)_2$ and $ZnSO_4$ according to the method described by Somogyi (1945). A 1 ml aliquot of dilute deproteinized plasma was evaporated *in vacuo* at 80° C until dryness (Altsuler et al.., 1975). The residue was reconstituted with 1 ml of distilled water, 10 ml of Scinti Verse I (Fisher Scientific, Fair Lawn, NJ) scintillation cocktail was added and the samples were counted in a Nuclear Chicago Mark II scintillation counter (Nuclear Chicago Corporation, Des Plain, IL.), set for ^{3}H, for 20 min at 4° C. Counting efficiency was determined using the internal standard technique.

C - The Determination of the Concentration of Plasma Free Fatty Acids ($mEq.l^{-1}$)

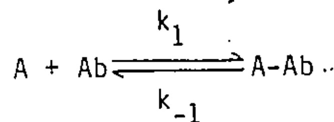
A colorimetric micromethod, based on the formation of FFA-Cu soaps, as described by Laurell and Tibbling (1966) was used. The chloroform-heptane (4:3 with 2% methanol) extraction of long chain fatty acids from 0.05 ml of plasma was carried out in the presence of silicic acid as a phospholipid trapping agent, to avoid possible contamination. A sodium chloride

saturated solution of cupric nitrate and triethanolamine (TEA) was used as a saponification reagent and 1,5-diphenylcarbohydrazide was used for the colorimetric determination of copper. The absorbance at 550 nm of each sample (in duplicate) and standards were measured on a Beckman DU-7 spectrophotometer (Beckman Instruments Inc., Fullerton, CA).

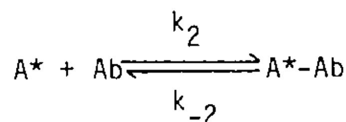
Special care was taken so that no solvent was lost by evaporation during the procedure, which might lead to falsely elevated readings.

D - The Determination of Immunoreactive Insulin (IRI) Concentration in the Plasma ($\mu U.ml^{-1}$)

The dextran coated charcoal method, for the insulin radioimmunoassay (IRA), described by Herbert et al.. (1968) was used for the first and third series. This method is based on the competitive binding principle. A homogenous population of hormone (antigen) reacts on a one to one basis with a fixed number of antibodies to form antigen-antibody complexes. If a portion of the hormone in the sample is labelled (i.e. "tagged" with ^{125}I), the reaction can be written as follows:



$$A + Ab \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A\text{-}Ab$$



$$A^* + Ab \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} A^*\text{-}Ab$$

where A is the antigen (hormone), A* is the labelled antigen, Ab is the antibody, A-Ab is the antigen-antibody complex, A*-Ab is the labelled antigen-antibody complex, k_1, k_2 are association rate constants and k_{-1}, k_{-2} are dissociation constants. It is assumed that $k_1 = k_2$ and that $k_{-1} = k_{-2}$. Therefore labelled and unlabelled antigens compete for a fixed number of antigen binding sites. Thus as the total amount of unlabelled antigen in the sample increases, less labelled antigen-antibody complex is formed (Campfeield at al, 1983). The amount of A*-Ab is measured in a gamma counter, and is related to the hormone concentration in the sample by the means of a standard curve.

The IRI assay was graciously carried out by Dr. Mladen Vranic and his group at the University of Toronto.

E - The Determination of Immunoreactive Glucagon (IRG) Concentration in the Plasma

Two IRA were used, the Faloona and Unger (1974) method using the 30 K antibody, for the first and third series, and the Heding (1971) method, using the K 5563 antibody (Novo Research), for the second series. These two assays are based on the same principle of competitive binding as the insulin IRA, which was described above. The Faloona method was kindly carried out in Toronto by Dr. Vranic's laboratory.

A kit for the determination of plasma pancreatic glucagon

based on the Heding method was purchased from Novo Research Institute, Denmark. Glucagon had to be extracted from the sample with 1.8 ml of 96% ethanol per 1 ml plasma. The mixture was shaken and centrifuged at 3,000 rpm (2,000 x g), the supernatant evaporated to dryness in vacuo (10 mm Hg pressure) and reconstituted in 1 ml of a phosphate-albumin buffer (pH 6.6 - 7.3). An aliquot of 0.1 ml of reconstituted extract or standard were incubated at 4° C for 24 hours with 0.1 ml of rabbit anti-(pork-glucagon) antiserum. The ^{125}I-pork-glucagon was added, and the mixture incubated for another 24 hours at 4° C. The bound and unbound hormone were separated by ethanol precipitation of the antigen-antibody complex. The precipitate was washed with 96% ethanol-buffer solution, centrifuged, the washing discarded, and the pellet was counted, dissolved in 0.5 ml of 0.05 N NaOH, in a LKB 1282 Compugamma gamma counter (LKB Wallac - Wallac, Oy, Turku, Finland). The plasma concentration was read from a curve plotting the fraction of bound labelled glucagon against concentration. The determination for standards was in triplicate, for unknowns in duplicate. The results were corrected for dilution due to the addition of preservatives to the blood sample.

F - The Determination of Plasma Norepinephrine, Epinephrine and Dopamine Concentration ($pg.ml^{-1}$)

The radioenzymatic assay described by Sole and Hussain

(1977) was carried out by Dr. Vranic's group in Toronto. This method simultaneously measures epinephrine, norepinephrine and dopamine. Plasma samples (0.05 ml) were incubated with catechol-O-methyl transferase and tritiated S-adenoxyl-methionine. The catecholamines were converted to O-methylated tritiated derivatives. These derivatives were isolated, after extraction, by one-way thin layer chromatography. The tritiated derivatives were scraped off the plate and counted. The concentration of the three catecholamine is proportional to the ratio of counts in the sample to that of the counts in a known standard.

VI Calculations

A - Glucose Kinetics: Rate of Appearance and Disappearance of Glucose

The rate of appearance of glucose (Ra) is equated to the hepatic glucose production or output. The rate of disappearance of glucose is equated to the rate of glucose utilization plus glycosuria.

The primed infusion method described by De Bodo (1964) based on the principle of tracer dilution was used to evaluate glucose kinetics using a one compartment model. Ra was calculated using Steele's equation (Steele, 1959).

$$Ra = \frac{R^* - pV \cdot x \frac{C_1 + C_2}{2} x \frac{SA_2 - SA_1}{t_2 - t_1}}{1/2\ (SA_1 + SA_2)}$$

where Ra is the rate of appearance of glucose ($mg.min^{-1}$), R* is the rate of infusion of labelled glucose ($dmp.min^{-1}$), p is the "pool fraction" - the rapidly mixing portion of the total glucose pool - taken to be 0.65 (Cowan and Hetenyi, 1971), V is the glucose distribution volume (ml), taken to be 220 $ml.kg^{-1}$ (Radziuk et al.., 1978), C_1 and C_2 are the plasma glucose concentrations ($mg.dl^{-1}$) and SA_1 and SA_2 are the specific activities ($dpm.mg^{-1}$) of glucose at time t_1 and t_2 (min), respectively. The rate of disappearance (Rd) is related to Ra in the following way (Steele, 1959):

$$Rd = Ra - pV \ x \ \frac{C_2 - C_1}{t_1 - t_2}$$

where Rd is the rate of disappearance of glucose ($mg.min^{-1}$). In this study Ra and Rd are normalized for body weight and have the units $mg.min^{-1}.kg^{-1}$.

B - Molar IRI/IRG Ratio

The radioimmunoassays give the concentration of insulin and glucagon in $\mu U.ml^{-1}$ and $pg.ml^{-1}$, respectively. The molar ratio is then given by the formula (Brekke et al., 1982):

$$IRI/IRG = \frac{[Insulin]\ (\mu U.ml^{-1})}{[Glucagon]\ (pg.ml^{-1})} \times 23.33$$

C - Statistical Analysis

Means and standard errors of the mean (SEM) were calculated for each measured and calculated quantity for each time point. The average value of each quantity for each phase of an experiment were compared using a two-way analysis of variance (factors are: phase and dog) (Snedecor, 1956). Changes with time in a phase were also evaluated in the same way (factors are: time and dog) followed by a Dunnett test (Dunnett, 1955) or Tukey test (Roscoe, 1975 and Keppel, 1973), for multiple comparisons of means, to determine significant differences with respect to control values. One sample t-test (Colton, 1974) were performed to determine significant differences of the relative value of the measured and calculated parameters when compared to control (100%).

The basal values for IRG and IRI concentrations and the IRI/IRG ratio of the three experiments of the first series were compared to normal nondiabetic controls using a one-way anlaysis of variance followed by a Dunnett test. Differences in basal FFA concentration in this series were determined by the Scheffe test for all possible comparisons (Roscoe, 1975). The basal values for all parameters, measured or calculated, of the second and third series, were compared by paired t-test (Colton, 1974).

Basal values for different treatements, i.e. normal (hormones only), alloxan-diabetes, treated diabetes,

pancratectomy, and methylprednisolone, were compared using a one-way analysis of variance and a Scheffe test. If individual pairwise comparison showed no significant difference, and that in our opinion it should, an unpaired t-test was carried out.

One sample t-tests, paired and unpaired t-test, as well as one-way and two-way analyses of variance were performed with the help of packaged programmes run on a Wang 600 minicomputer.

VII Chemicals

The [3-^{3}H]-glucose was purchased from New England Nuclear Canada (Lachine, Que., Canada). Alloxan monohydrate, phlorizin, aprotinin, human albumin and thimerosal were obtained from Sigma Chemical Company (St. Louis, MO). Injectable crystalline and protamine zinc beef and pork insulins were supplied by Connaught Laboratories Limited (Willowdale, Ont., Canada). Glutathione and streptozotocin were purchased from Boehringer Mannheim Canada (Dorval, Que., Canada). Methylprednisolone acetate (Depo-Medrol) was supplied by the Upjohn Company of Canada (Don Mills, Ont., Canada). Procine pancreatic enzymes (Viokase-V) were purchased from A-H Robins Canada Inc. (Montreal, Que., Canada). Anti-(beef insulin) antiserum and the 30 K antiserium were supplied by Dr. Peter Wright (Indianapolis, IN) and Dr. Roger Unger (Dallas, TX). respectively. ^{125}I-labelled pork insulin, ^{125}I-labelled glucagon and the glucagon IRA kit were obtained from the Novo Research

Institute (Copenhagen, Denmark). All chemicals were of the highest grade of purity available.

CHAPTER III

RESULTS

I The Basal States

Dogs were observed in five basal states: namely the normal nondiabetic, the alloxan-diabetic, the alloxan-diabetic treated with insulin (treated-diabetic), the pancreatectomized-diabetic and the methylprednisolone treated (MP-treated) states.

The plasma concentration of IRG was determined in all five states, that of IRI and the plasma IRI/IRG ratio for every state except in pancreatectomized dogs. Glucose concentration, Ra, and Rd were determined in all states except in normal dogs. Basal FFA concentration was determined in treated and non-treated alloxan-diabetic dogs as well as in dogs treated with methylprednisolone (Table 1).

IRG concentration in the plasma of alloxan diabetic dogs was significantly above that of normals ($F = 19.400$, $p < 0.001$). The plasma IRG level in the other groups did not differ from normals or each other. Accordingly alloxan-diabetic dogs had a higher IRG level than the treated-diabetic, the pancreatectomized and the MP-treated dogs ($F = 8.367$, $p < 0.001$, $F = 6.053$, $p < 0.005$ and $F = 6.350$, $p < 0.005$ respectively).

The basal concentration of IRI in MP-treated dogs, and

Table 1

Basal values for measured and calculated parameters in the five basal steady states studied.

Parameter	Normal	Alloxan-Diabetic	Treated-Diabetic	Pancrex	MP
IRG ($pg \cdot ml^{-1}$)	71 ± 12	440 ± 81	59 ± 6	93 ± 15	85 ± 11
IRI ($\mu U \cdot ml^{-1}$)	11 ± 1	5.8 ± 0.4	18 ± 3	-	22 ± 3
Glucose ($mg \cdot dl^{-1}$)	-	324 ± 23	90 ± 21	394 ± 31	110 ± 1
FFA ($mEq \cdot l^{-1}$)	-	2.2 ± 0.2	0.8 ± 0.2	-	1.6 ± 0.2
Ra ($mg \cdot min^{-1} \cdot kg^{-1}$)	-	5.5 ± 0.4	3.5 ± 0.4	3.3 ± 0.4	4.5 ± 0.5
Rd ($mg \cdot min^{-1} \cdot kg^{-1}$)	-	6.1 ± 0.5	3.1 ± 0.3	3.8 ± 0.5	4.8 ± 0.5
IRI/IRG (molar)	4.4 ± 0.6	0.45 ± 0.13	8.1 ± 2.1	-	6.8 ± 1.6

Means ± SEM are shown.

insulin treated diabetic dogs was significantly above that observed in all other animals. As expected, the alloxan-diabetic dogs had the lowest concentration of insulin. The peripheral basal insulin of the insulin treated diabetic dogs was significantly above that of normal animals ($F = 3.664$, $p < 0.05$).

Glucose concentration in the plasma was highest in the pancreatectomized group (394 ± 31 $mg.dl^{-1}$), however, this was not significantly different from the basal value of the alloxan-diabetic dogs ($F = 1.485$, n.s.). The lowest glucose level was recorded in treated-diabetic animals and was significantly lower than that of either pancreatectomized or alloxan-diabetic dogs ($F = 18.354$, $p < 0.001$ and $F = 13.901$, $p < 0.001$), however, it was similar to that of the MP-treated subjects.

Alloxan diabetic dogs, with a mean plasma FFA concentration of 2.2 ± 0.2 $mEq.l^{-1}$, had a significantly higher concentration than treated-diabetic dogs ($F = 11.542$, $p < 0.001$). The plasma FFA level in MP-treated dogs was also significantly above ($t = 2.545$, $p < 0.05$) that in insulin treated animals, but did not differ from that in diabetic dogs ($F = 2.088$, n.s.).

Alloxan-diabetic dogs had the higest Ra, which was significantly higher than that measured in pancreatectomized or treated-diabetic animals ($F = 4.292$, $p < 0.025$ and $F = 3.462$, $p < 0.05$ respectively). The difference between the latter two groups was not significant ($F = 0.033$, n.s.). The Ra of the MP-treated animal was similar to that of the alloxan-diabetic dog

($F = 0.820$, n.s.), however it was not significantly different from that in the pancreatectomized and insulin treated diabetic animals ($F = 1.315$, n.s. and $F = 1.019$, n.s. respectively).

No significant difference could be detected between the basal Rd in the alloxan-diabetic and the MP-treated dogs, neither between the pancreatectomized and the treated-diabetic states. The alloxan-diabetic dogs had a higher Rd than either the pancreatectomized or the treated-diabetic dogs ($F = 3.526$, $p < 0.05$ and $F = 6.886$, $p < 0.005$). However, the MP-treated group had a significantly higher Rd than the treated-diabetic group ($t = 2.939$, $p < 0.05$) while they had a similar Rd to that of the pancreatectomized dogs.

The basal IRI/IRG ratio was the lowest in alloxan-diabetic dogs at a level of 0.45 ± 0.13. The ratio in diabetic dogs was significantly less than in normals ($t = 5.92$, $p < 0.001$) or MP-treated and treated-diabetic animals ($F = 4.991$, $p < 0.01$ and $F = 14.093$, $p < 0.001$ respectively). The highest ratio (8.1 ± 2.1) was found in insulin treated alloxan diabetic animals. This was statistically different from that observed in normals ($t = 2.426$, $p < 0.05$), but not in MP-treated animals ($F = 1.149$, n.s.).

II The First Series - Alloxan Diabetic Dogs

A - Type I Experiments - Alloxan-diabetic Dogs: Responses to the Infusion of Phlorizin

The results of Type I experiments are shown on Figure 1.

Figure 1: Effects of an infusion of phlorizin (50 $\mu g.kg^{-1}.min^{-1}$) to 7 insulin deprived alloxan-diabetic dogs on plasma concentration of glucagon (IRG), insulin (IRI), glucose, rate of glucose appearance (Ra) and disappearance (Rd). Abscissa: time in min. Left ordinates: variables in absolute units. Right ordinates: variables expressed as a percentage of their control value. Standard errors of the mean are shown as vertical bars. IRG: 100% = 464 ± 121 $pg.ml^{-1}$, IRI: 100% = 5.5 ± 0.5 $\mu U.ml^{-1}$, Plasma glucose: 100% = 328 ± 29 $mg.dl^{-1}$, Ra: 100% = 5.73 ± .79 $mg.min^{-1}.kg^{-1}$, Rd: 100% = 6.4 ± 1.0 $mg.min^{-1}.kg^{-1}$.

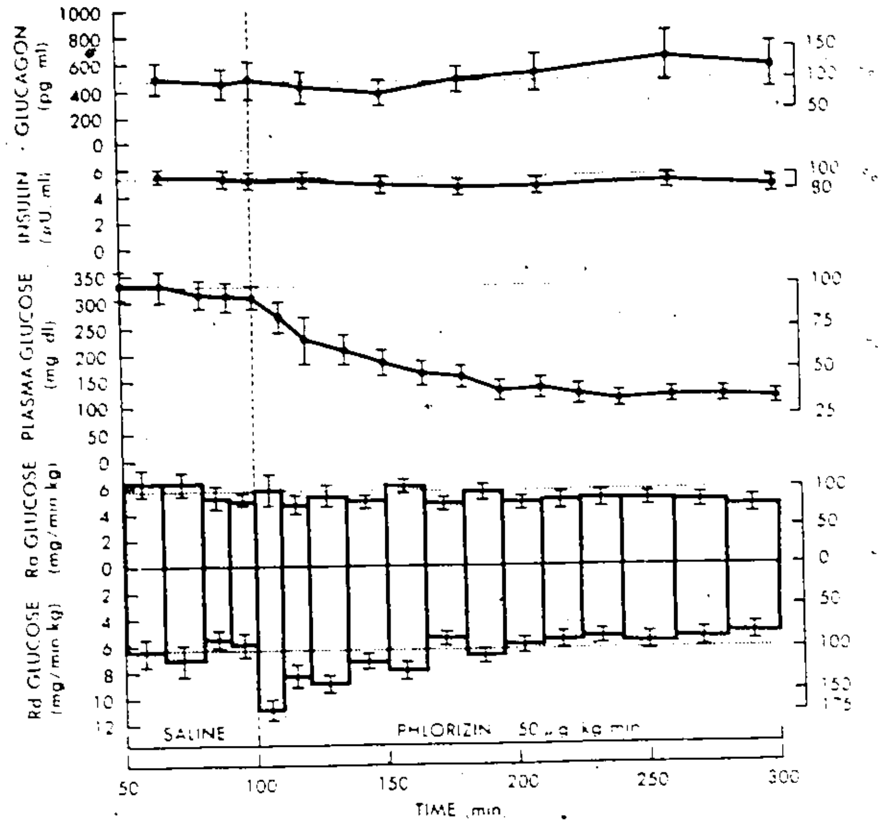


Figure 1

In the control (basal) period, prior to the infusion of phlorizin, the level of plasma glucose was 328 ± 29 $mg.dl^{-1}$. During the infusion plasma glucose fell monotonically and reached 114 ± 16 $mg.dl^{-1}$ by t = 300 min ($t = 24.03$, $p < 0.0005$). The basal IRI concentration was 5.5 ± 0.5 $\mu U.ml^{-1}$, significantly below 11 ± 1 $\mu U.ml^{-1}$ found in the same dog before the injection of alloxan. During the infusion, plasma IRI decreased significantly by t = 180 min ($t = 2.366$, $p < 0.05$) and stayed at this low level until the end of the experiment. The basal Ra was 5.73 ± 0.79 $mg.min^{-1}.kg^{-1}$ significantly above the range observed in normal dogs (Gauthier et al., 1980; Gauthier and Hetenyi, 1982; Gauthier et al., 1983 and Hetenyi et al., 1983). During the infusion of phlorizin Ra decreased to 4.7 ± 0.5 $mg.min^{-1}.kg^{-1}$ ($t = 2.704$, $p < 0.05$) and remained about this level until the end of the experiment. The Rd increased significantly from its basal level of 6.4 ± 1.0 $mg.min^{-1}.kg^{-1}$ to a peak of 10.9 ± 0.8 $mg.min^{-1}.kg^{-1}$ during the first 10 minutes of the infusion ($t = 4.234$, $p < 0.005$), then, in parallel with the falling concentration of plasma glucose, waned to 5.08 ± 0.69 $mg.min^{-1}.kg^{-1}$, significantly below its basal value ($t = 2.861$, $p < 0.025$) by the end of the experiment.

Basal plasma IRG was 464 ± 121 $pg.ml^{-1}$ significantly above 72 ± 12 $pg.ml^{-1}$ ($t = 4.159$, $p < 0.01$), the level found in the same dog before the injection of alloxan. During the

Table 2

The plasma concentration of IRG and glucose before and during the last 90 minutes of an infusion of 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ phlorizin

Experiment	Mean basal level of IRG	Mean basal level of Glucose	Concentration during the infusion of phlorizin: t = 210 IRG	t = 210 Glucose	t = 280 IRG	t = 280 Glucose	t = 300 min IRG	t = 300 min Glucose
2	531 ± 42	230 ± 3	1109*	96	1449*	79	-	-
3	165 ± 18	327 ± 7	319*	120	337*	110	401*	96
10	506 ± 11	402 ± 12	267	190	348	157	499	141
14	545 ± 16	390 ± 7	641	214	562	184	712*	161
17	952 ± 47	313 ± 4	723	100	1032	79	1193	72
21	120 ± 13	345 ± 3	149	138	178	115	143	101

Plasma IRG concentrations marked by an asterisk are above the upper limit of the 95% confidence interval of the mean basal level. All concentrations of plasma glucose at $t > 210$ min are below the lower limit of the 1% confidence interval of the mean basal level. For the basal levels mean ± S.E.M. are shown. Concentration of IRG in $pg \cdot ml^{-1}$ of glucose in $mg \cdot dl^{-1}$.

Figure 2: Upper panel: Effect of an infusion of phlorizin (50 $\mu g.kg^{-1}.min^{-1}$) to 5 insulin deprived (upper line) and 5 insulin treated (lower line) alloxan-diabetic dogs on plasma concentration of free fatty acids (FFA). Lower panel: Effect of an infusion of insulin (7 $mU.kg^{-1}.min^{-1}$) to 5 insulin deprived alloxan-diabetic dogs on the concentration of FFA. Abscissa: time in min. Ordinate: Absolute concentration in $mEq.l^{-1}$. Standard errors of the mean are shown as vertical bars.

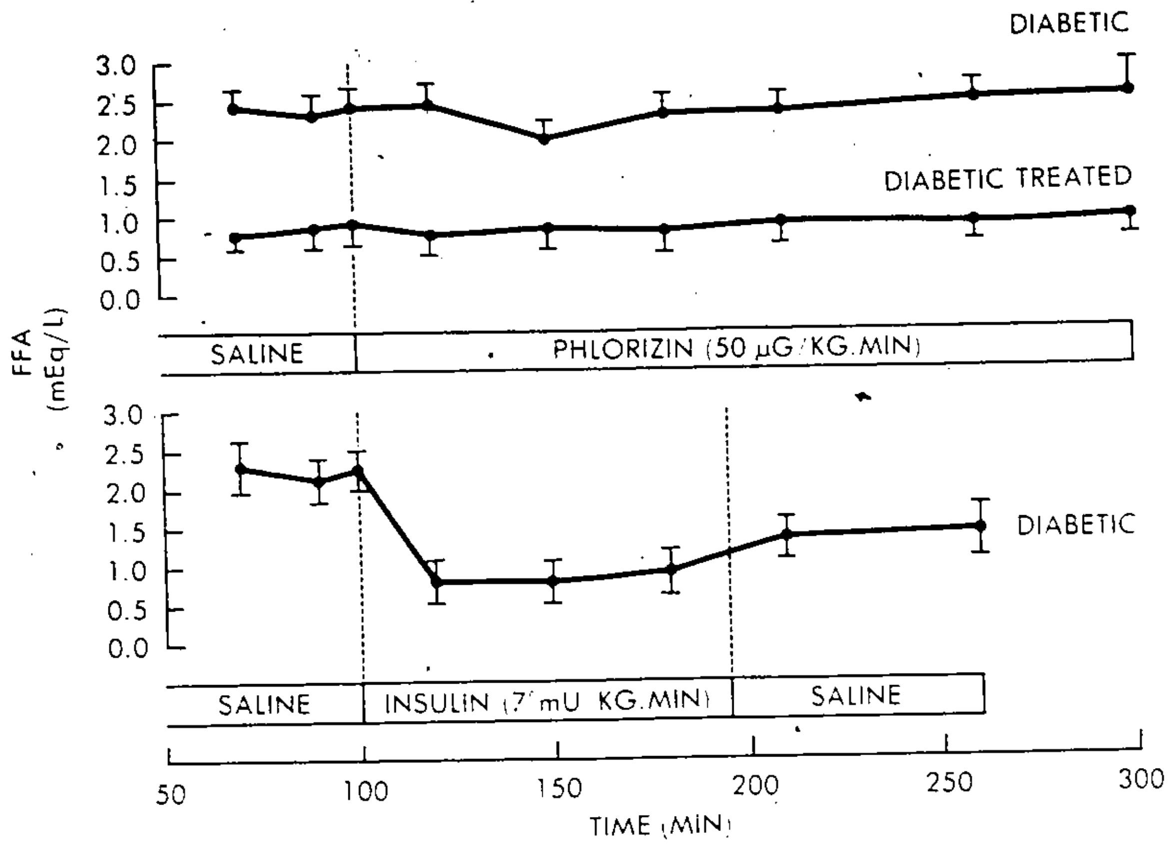


Figure 2

infusion of phlorizin in three out of six experiments plasma IRG rose significantly above the upper limit of the 95 % confidence interval of the mean basal value for those experiments. In the other three experiments plasma IRG did not change (Table 2).

The basal plasma FFA concentration was 2.40 ± 0.26 $mEq.l^{-1}$. No significant change was observed during the phlorizin infusion (Figure 2).

During the infusion no change in the level of plasma epinephrine was noted (Table 3). Plasma norepinephrine concentration was slightly elevated by the end of the experiment. Plasma dopamine concentration tended to decrease, however, the decrease was significant only at t = 120 min (i.e. 20 min after the commencement of the infusion).

The molar insulin to glucagon ratios are shown on Table 4. The basal ratio is about 11% of that observed in the same dog prior to the alloxan injection. During the phlorizin infusion the ratio was unchanged (F = 0.440, n.s.).

B - Type II Experiments - Insulin Treated Alloxan-diabetic Dogs: Responses to the Infusion of Phlorizin

The results of Type II experiments are summarized on Figure 3. The basal level of plasma glucose was 90 ± 20 $mg.dl^{-1}$ and did not change significantly during the infusion of phlorizin. Plasma IRI was significantly above the level found in the same

Table 3

Plasma concentrations of Epinephrine (E), Norepinephrine (NE) and Dopamine (D) in the plasma of four alloxan-diabetic dogs, during the infusion of 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ phlorizin (Type I) or 7 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin (Type III)

Infusion (Expt)	BASAL LEVEL	During/After Infusion at t = (min)					
		120	150	180	210	260	300
PHLORIZIN (Type I)							
E	70 ± 11	152 ± 72	45 ± 10	64 ± 22	86 ± 19	108 ± 41	150 ± 38
NE	164 ± 34	238 ± 40	134 ± 25	187 ± 13	186 ± 46	201 ± 77	280 ± 66*
D	86 ± 9	75 ± 9*	77 ± 14	70 ± 50	81 ± 18	72 ± 13	89 ± 7
INSULIN (Type III)**							
E	109 ± 36	211 ± 152	214 ± 104*	298 ± 104*	433 ± 201	292 ± 117*	-
NE	234 ± 73	222 ± 96	208 ± 64	250 ± 73	367 ± 145	313 ± 97	-
D	71 ± 5	56 ± 9	60 ± 10	95 ± 32	74 ± 25	77 ± 13	-

Mean values ± S.E.M. are shown, as pg/ml.

*The mean change from the basal value is significant ($P < 0.05$).
**Insulin was infused between t = 100 and 195 minutes.

Figure 3: Effect of an infusion of phlorizin (50 $\mu g.kg^{-1}.min^{-1}$) to 5 insulin treated alloxan-diabetic dogs on plasma concentration of glucagon (IRG), insulin (IRI), glucose, rate of glucose appearance (Ra) and disappearance (Rd). Abscissa: time in min. Left ordinates: variables in absolute units. Right ordinates: variables expressed as a percentage of their control value. Standard errors of the mean are shown as vertical bars. IRG: 100% = 59 ± 5 $pg.ml^{-1}$, IRI: 100% = 18155 ± 3.0 $\mu U.ml^{-1}$, Plasma glucose: 100% = 90 ± 20 $mg.dl^{-1}$, Ra: 100% = 3.55 ± .36 $mg.min^{-1}.kg^{-1}$, Rd: 100% = 3.03 ± .35 $mg.min^{-1}.kg^{-1}$.

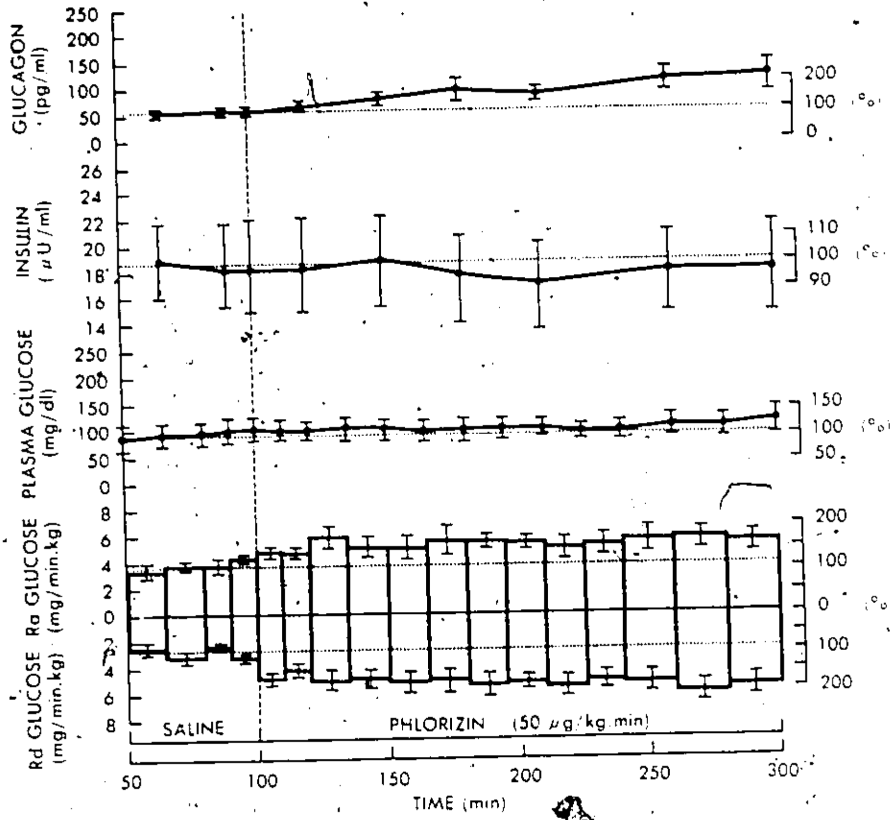


Figure 3

dog before the injection of alloxan ($F = 3.664$, $p < 0.05$). During the infusion of phlorizin the mean concentration of plasma IRI decreased during the course of the experiment. The minimal level was reached by t = 210 min ($t = 2.141$, $p < 0.05$), further changes were not significant. Ra increased by 50 ± 16% ($F = 43.55$, $p < 0.001$) and Rd by 71 ± 22% ($F = 150.3$, $p < 0.001$). The concentration of plasma IRG increased monotonically from 59 ± 5 $pg.ml^{-1}$ to 110 ± 32 $pg.ml^{-1}$ by t = 300 min ($F = 15.337$, $p < 0.001$).

The average basal level of FFA in the plasma of the insulin treated dog in this experiment was significantly lower ($q = 7.823$, $p < 0.001$) than of the Type I experiments at 0.819 ± 0.22 $mEq.l^{-1}$.

In the insulin treated dogs the basal IRI/IRG ratio was 1.84 times that found in the same dogs in the normal state, and did not change during the phlorizin infusion ($F = 0.180$, n.s.).

C - Type III Experiments - Alloxan-diabetic Dogs: Responses to i.v. Infused Insulin

The infusion of insulin decreased the concentration of plasma glucose from an average of 338 ± 5 to 74 ± 24 $mg.dl^{-1}$ in 95 minutes (Figure 4). The mean initital level did not differ significantly from that observed in Type I experiments ($t = 0.277$, n.s.). When the infusion of insulin was changed to

Figure 4: Effect of an infusion of insulin ($7 mU.kg^{-1}.min^{-1}$) to 5 insulin deprive alloxan-diabetic dogs on plasma concentration of glucagon (IRG), insulin (IRI), glucose, rate of glucose appearance (Ra) and disappearance (Rd). Abscissa: time in min. Left ordinates: variables in absolute units. Right ordinates: variables as a percentage of their control value. Standard errors of the mean are shown as vertical bars. IRG: 100% = 379 ± 108 $pg.ml^{-1}$, IRI: 100% = 5.9 ± 0.6 $\mu U.ml^{-1}$, Plasma glucose: 100% = 338 ± 5 $mg.dl^{-1}$, Ra: 100% = 5.62 ± .38 $mg.min^{-1}.kg^{-1}$, Rd: 100% = 6.16 ±.28 $mg.min^{-1}.kg^{-1}$.

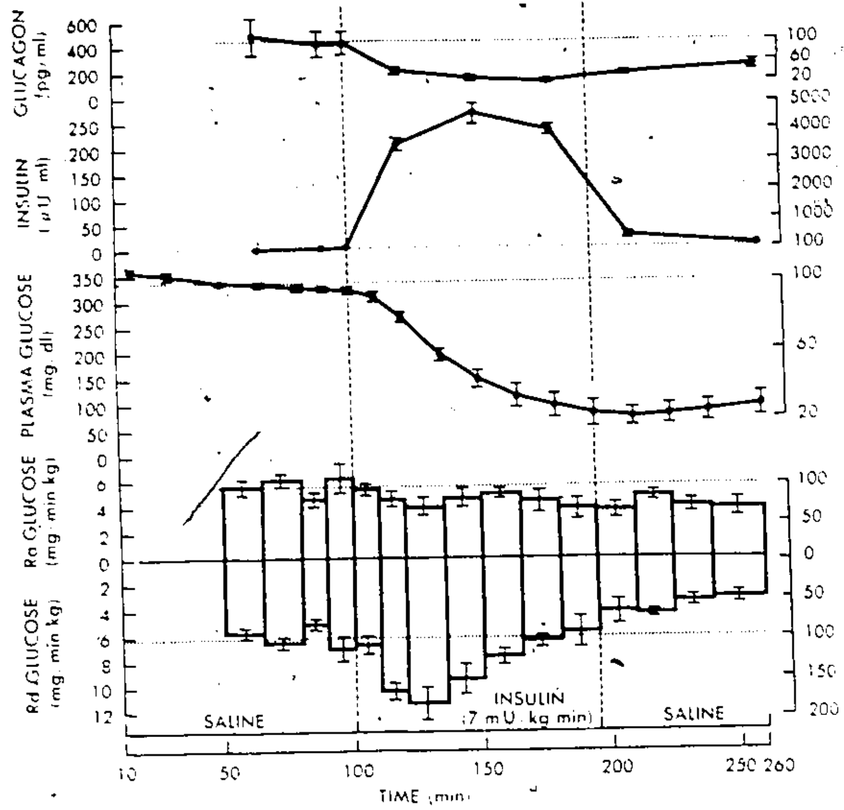


Figure 4

saline, no significant rise in plasma glucose was observed within 65 minutes ($F = 0.17$, n.s.). Glucose production (Ra) was reduced during the infusion of insulin from its basal 5.6 ± 0.4 $mg.min^{-1}.kg^{-1}$ to a minimum of 4.1 ± 0.7 $mg.min^{-1}.kg^{-1}$ which was reached between $t = 120$ and 135 min. The difference was significant ($F = 5.92$, $p < 0.025$). Rd rose during the infusion of insulin to a maximum of 11 ± 1 $mg.min^{-1}.kg^{-1}$ ($t = 4.046$, $p < 0.025$), but tended to decrease as the concentration of glucose fell. By the end of the experiment Rd was below its pre-infusion level ($F = 40.31$, $p < 0.001$) although the concentration of plasma insulin was still above its pre-infusion level ($F = 5.92$, $p < 0.025$).

The concentration of plasma IRG dropped from its initial level of 379 ± 108 $pg.ml^{-1}$ to 151 ± 33 $pg.ml^{-1}$ within 20 minutes of the start of the insulin infusion ($t = 8.635$, $p < 0.0005$). Similarly to plasma glucose, the concentration of IRG did not rise significantly within the 65 minutes after the end of the infusion of insulin ($F = 1.564$, n.s.).

The basal value of FFA concentration was not significantly different than that for Type I experiments ($q = 0.84$, n.s.), however, it was significantly lower than for Type II experiments ($q = 6.981$, $p < 0.01$). The infusion of insulin caused a $65 \pm 9\%$ decrease by $t = 120$ min ($t = 6.892$, $p < 0.0005$), from which no recovery was noted during the 65 minutes of a

Table 4

Molar ratio of IRI/IRG in plasma before and during the infusion of 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ phlorizin or 7 $mU^{-1} \cdot min^{-1}$ insulin.
Mean ratio before the injection of alloxan = 4.38 ± 0.57.

Expt.	Before* infusion basal	During and after infusion at t = (min)					
		120	150	180	210	260	300
TYPE I diabetic	·47 ± ·17 (6)	·46 ± ·15 (6)	·50 ± ·17 (6)	·29 ± ·07 (6)	·37 ± ·13 (6)	·32 ± ·11 (6)	·30 ± ·11 (5)
TYPE II diabetic treated	8.1 ± 2.1 (5)	7.8 ± 2.2 (5)	7.0 ± 2.0 (5)	6.7 ± 2.8 (5)	5.8 ± 1.7 (5)	6.0 ± 2.1 (4)	5.9 ± 2.0 (4)
TYPE III diabetic	·52 ± ·17	43 ± ·6	79 ± 13	84 ± 19	5.8 ± 1.5	1.8 ± ·5	- -

In Type I and II experiments phlorizin was infused between t = 100 and 300 minutes.
In Type III experiments insulin was infused between t = 100 and 195 minutes.
*All basal values are significantly ($P < 0.05$) different from normal (4.38 ± 0.57) as tested by Dunnet's test.

consecutive infusion of saline.

During the infusion of insulin the concentration of epinephrine rose significantly (Table 3). No changes in the concentration of norepinephrine and dopamine were observed.

The insulin/glucagon ratio was increased by one order of magnitude (Table 4) during the infusion period.

III Second Series - Pancreatectomized Dogs

A - Responses to the Infusion of Phlorizin

The results of these experiments are summarized on Figure 5. Basal glucagon (IRG) concentration was 103 ± 13 $pg.ml^{-1}$. Basal plasma glucose level was 402 ± 26 $mg.dl^{-1}$. The rate of glucose production (Ra) and of disappearance (Rd) were 3.1 ± 0.6 $mg.min^{-1}.kg^{-1}$ and 3.7 ± 0.7 $mg.min^{-1}.kg^{-1}$ respectively.

During the infusion of phlorizin, the IRG concentration increased ($F = 9.801$, $p < 0.005$), the absolute increase was significant at $t = 120$ ($p < 0.05$), $t = 260$ ($p < 0.01$) and $t = 300$ ($p < 0.05$) min while the relative increase was significant as of $t = 150$ until the end of the infusion. Glucose concentration dropped monotonically during the whole infusion period ($F = 156.8$, $p < 0.001$). Ra remained unchanged, however, in two out of four experiments glucose production increased to a level above the upper limit of the 95% confidence interval of the mean basal value for those experiments. The increase was transient and was not maintained

Figure 5: Effects of an infusion of phlorizin (50 $\mu g.kg^{-1}.min^{-1}$) to 4 pancreatectomized dogs on the plasma concentration of glucagon (IRG), glucose, rate of glucose appearance (Ra) and disappearance (Rd). Abscissa: time in min. Left ordinates: variables in absolute units. Right ordinates: variable expressed as a percentage of their control value. Standard errors of the mean are shown as vertical bars. IRG: 100% = 103 ± 18 $pg.ml^{-1}$, Plasma glucose: 100% = 402 ± 21 $mg.dl^{-1}$, Ra: 100% = 3.1 ± 0.6 $mg.min^{-1}.kg^{-1}$, Rd: = 3.7 ± 0.7 $mg.min^{-1}.kg^{-1}$.

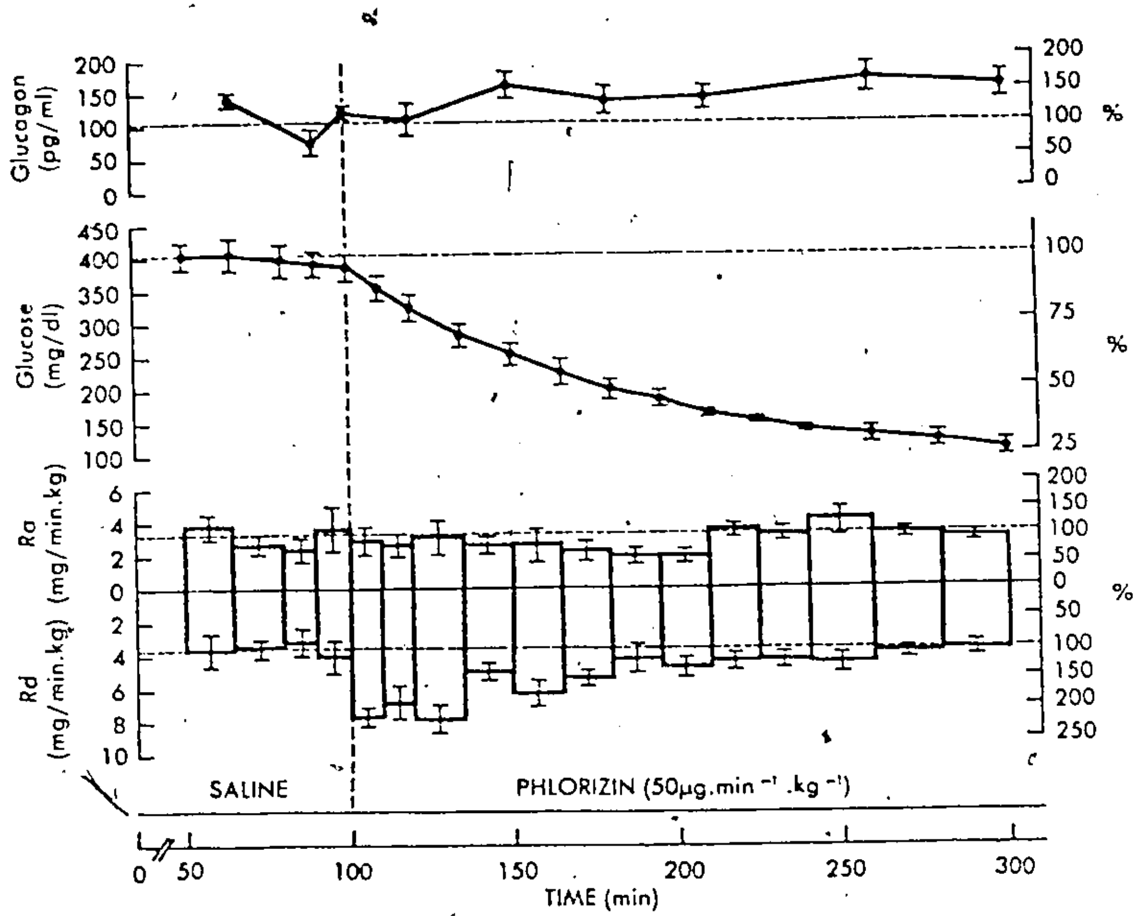


Figure 5

for more than 45 minutes. During the period of increased Ra, plasma glucose concentration tended to level off. Rd increased transiently ($F = 15.721$, $p < 0.001$) reaching a maximum of 7.9 ± 0.9 $mg.min^{-1}.kg^{-1}$ between t = 120 and 135 min and returned to basal by t = 195 min.

B - Responses to i.v. Infused Insulin

The results of these experiments are summarized on Figure 6. Basal plasma concentration of IRG was 85 ± 18 $pg.ml^{-1}$, of glucose 385 ± 45 $mg.dl^{-1}$. Basal Ra and Rd were 3.5 ± 0.5 $mg.min^{-1}.kg^{-1}$ and 3.9 ± 0.7 $mg.min^{-1}.kg^{-1}$ respectively.

During the infusion of insulin glucagon concentration remained constant. Plasma glucose dropped ($F = 68.1$, $p < 0.001$) and reached a minimum of 65 ± 12 $mg.dl^{-1}$ by the end of the period. Ra decreased transiently ($F = 10.33$, $p < 0.005$) and returned to the pre-infusion level by the end of the insulin infusion. Rd increased ($F = 23.79$, $p < 0.001$) and reached a peak of 9.9 ± 0.7 $mg.min^{-1}.kg^{-1}$ ($p < 0.01$) between t = 120 and 135 min, then returned to its basal level (4.9 ± 1.0 $mg.min^{-1}.kg^{-1}$) by t = 195 min.

After the cessation of the infusion of insulin, the plasma concentration of IRG and glucose remained low ($F = 0.087$, n.s. and $F = 1.337$, n.s respectively) and no change in Ra was observed ($F = 0.415$, n.s.). Rd decreased ($F = 6.501$, $p < 0.01$) to reach a minimum of 2.2 ± 0.3 $mg.min^{-1}.kg^{-1}$ by the

Figure 6: Effect of an infusion of insulin ($7mU.min^{-1}.kg^{-1}$) to 4 pancreatectomized dogs on the plasma concentration of glucagon (IRG), glucose, rate of glucose appearance (Ra) and disappearance (Rd). Abscissa: time in min. Left ordinates: variables in absolute units. Right ordinates: variables expressed as a percentage of their control value. Standard errors of the mean are shown as vertical bars. IRG: 100% = 85 ± 18 $pg.ml^{-1}$, Plasma clucose: 100% = 386 ± 45 $mg.dl^{-1}$, Ra: 100% = 3.5 ± .5 $mg.min^{-1}.kg^{-1}$, Rd: 100% = 3.9 ± .7 $mg.min^{-1}.kg^{-1}$.

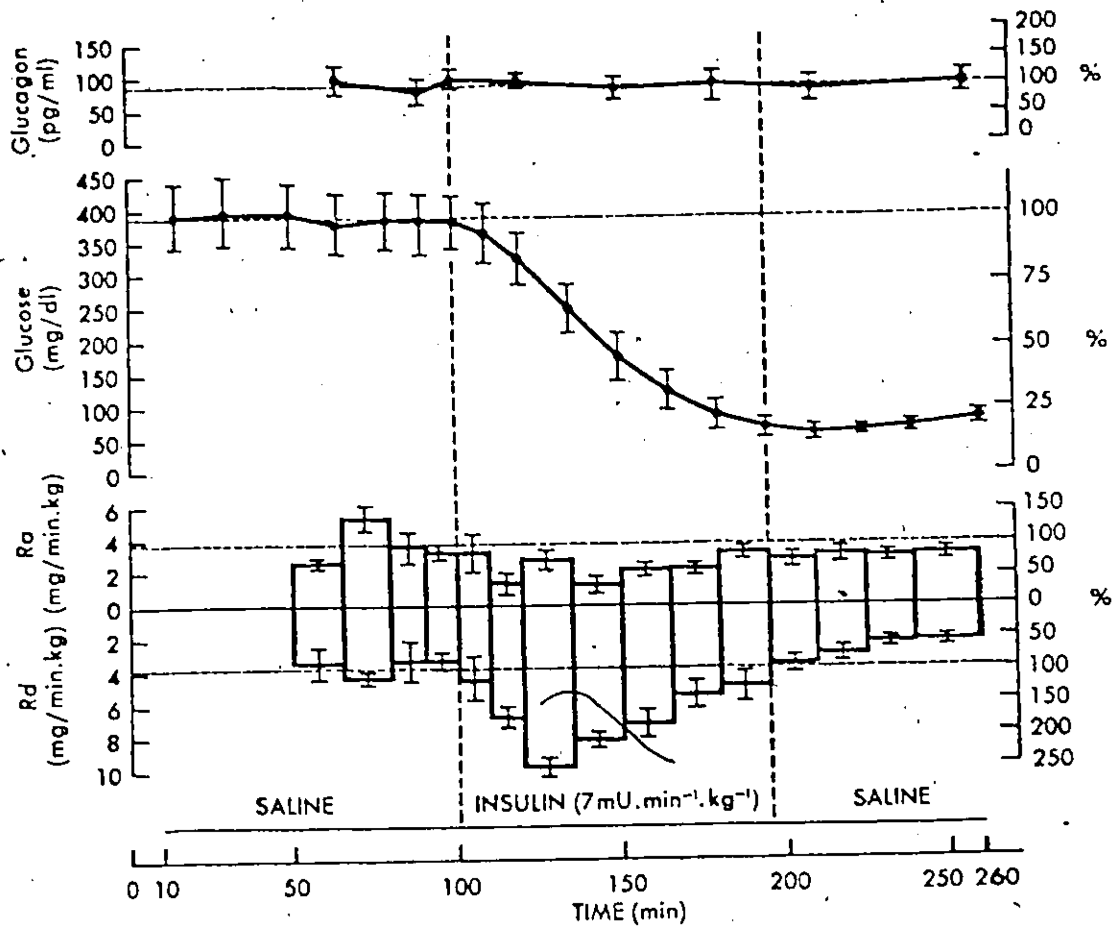


Figure 6

end of the experiment.

IV The Third Series - Methylprednisolone Treated Dogs

A - Responses to the Infusion of Phlorizin

The results of these experiments are summarized on Figure 7. The basal level of glucose in the plasma was 110 ± 1 $mg.dl^{-1}$, that of FFA, IRI and IRG: 1.50 ± 0.14 $mEq.l^{-1}$, 18.0 ± 0.8 $\mu U.ml^{-1}$, and 79 ± 12 $pg.ml^{-1}$ respectively. The IRI/IRG molar ratio was 5.68 ± 0.91 (Table 5). The rate of glucose production (Ra) was 5.15 ± 0.47 $mg.min^{-1}.kg^{-1}$, whereas Rd was found to be 5.44 ± 0.65 $mg.min^{-1}.kg^{-1}$.

During the infusion of phlorizin the concentration of glucose fell transiently for the first 10 to 15 minutes of the infusion ($p < 0.01$ at t = 110 nim). After t = 120 min the mean concentration was not different from basal. No significant change in the plasma FFA level was observed. Ra increased significantly ($F = 16.82$, $p < 0.001$) as calculated over the entire length of the infusion. It is noteworthy, however, that the increase became evident only after t = 110 min. Rd rose ($F = 27.25$, $p < 0.001$) to about the same degree as Ra. There was no significant change in the concentration of plasma IRI, although a tendency to decrease during the final half hour of the infusion was noticeable. Plasma IRG rose by about 25%. This was not significantly different from the pre-infusion level ($F = 2.099$, n.s.). The IRI/IRG ratio was consequently

Figure 7: Effects of an infusion of phlorizin (50 $\mu g.kg^{-1}.min^{-1}$) to 4 methylprednisolone treated dogs (3 $mg.kg^{-1}.day^{-1}$) on plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA), glucose , rate of glucose appearance (Ra) and disappearance (Rd). Abscissa: time in min. Left ordinates: variables in asolute units. Right ordinates: variables expressed as a percentage of their control value. Standard errors of the mean are shown as vertical bars. IRI: 100% = 79 ± 12 $pg.ml^{-1}$, IRI: 100% = 17.9 ± 08 $\mu U.ml^{-1}$, FFA; 100% = 1.5 ± .1 $mEq.l^{-1}$, Plasma glucose: 100% = 110 ± 1 $mg.dl^{-1}$, Ra: 100% = 5.2 ± .5 $mg.min^{-1}.kg^{-1}$, Rd: 100% = 5.4 ± .7 $mg.min^{-1}.kg^{-1}$.

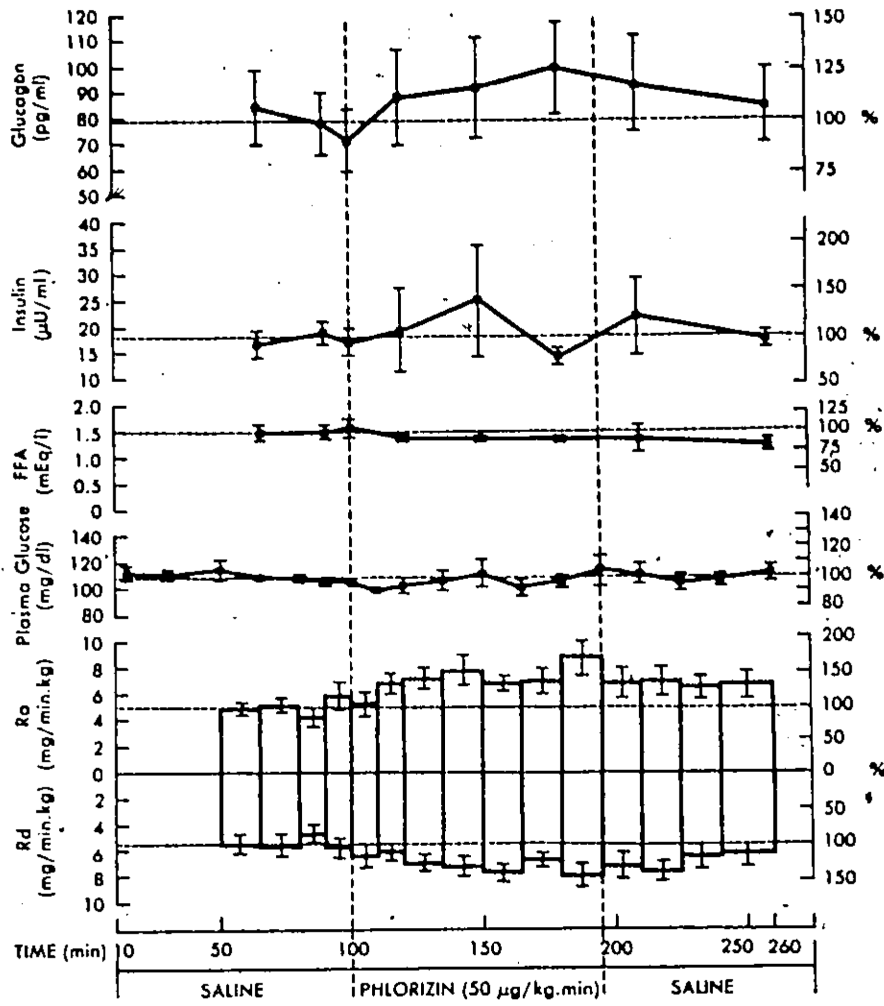


Figure 7

Table 5

Molar IRI/IRG ratio in methylprednisolon treated dogs before and during the infusion of 50 $\mu g \cdot min^{-1} \cdot kg^{-1}$ phlorizin or 7 $mU^{-1} \cdot min^{-1}$ insulin

TIME	Basal	Infusion			Recovery	
		120	150	180	210	260
Phlorizin	5.68 ± 0.91	4.98 ± 1.37	6.12 ± 1.52	3.54 ± 0.47	5.39 ± 1.26	5.18 ± 0.80
Insulin	7.96 ± 3.15	51.28 ± 6.84*	40.05 ± 37.9*	38.71 ± 3.094*	6.40 ± 1.70	6.41 ± 2.05

Basal conditions were n.s. different

*Significantly different from basal ($P < 0.01$).

not statisticaly different.

After changing the infusion from phlorizin to saline at t = 195 min, all the measured or calculated values remained unchanged.

B - Responses to i.v. Infused Insulin

The results of these experiments are shown on Figure 8. The basal level of glucose (112 ± 2 $mg.dl^{-1}$), FFA (1.71 ± 0.28 $mEq.l^{-1}$), IRI (26.3 ± 6.3 $\mu U.ml^{-1}$), IRG (91 ± 11 $pg.ml^{-1}$) were not significantly different from their respective counterparts (observed in the other experiment on the same animal). The basal Ra was 3.92 ± 0.43 $mg.min^{-1}.kg^{-1}$ and not significantly smaller than in the parallel experiments, Rd was 4.12 ± 0.35 $mg.min^{-1}.kg^{-1}$ and marginally smaller ($p < 0.05$) than its counterpart. The basal IRI/IRG ratio was 7.96 ± 3.15 (Table 5) and was not significantly different than in the parallel experiment.

During the infusion of insulin the concentration of glucose dropped to a minimum of 58.6 ± 3.4 $mg.dl^{-1}$ by t = 165 min. A small recovery was noticeable during the final 15 minutes of the infusion. The concentration of FFA fell significantly ($F = 8.373$, $p < 0.005$) but transiently and regained its basal level by t = 150 min ($t = 0.820$, n.s.). Ra and Rd both increased significantly as calculated over the entire period of the infusion ($F = 9.871$, $p < 0.005$ and $F = 19.98$, $p < 0.001$ respectively), but the increase in the former

Figure 8: Effects of an infusion of insulin ($7mU.kg^{-1}.min^{-1}$) to 4 methylprednisolone treated dogs ($3mg.kg^{-1}.day^{-1}$) on the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA), glucose, rate of glucose appearance (Ra) and disappearance (Rd). Abscissa: time in min. Left ordinates: variable in absolute units. Right ordinates: variables expressed as a percentage of their control value. Standard errors of the mean are shown as vertical bars. IRG: 100% = 91 ± 11 $pg.ml^{-1}$, IRI: 100% = 26 ± 6 $\mu U.ml^{-1}$, FFA: 100% = 1.7 $mEq.l^{-1}$, Plasma glucose: 100% = 112 ± 2 $mg.dl^{-1}$, Ra: 100% = 3.9 ± .4 $mg.min^{-1}.kg^{-1}$, Rd: 100% = 4.1 ±.3 $mg.min^{-1}.kg^{-1}$.

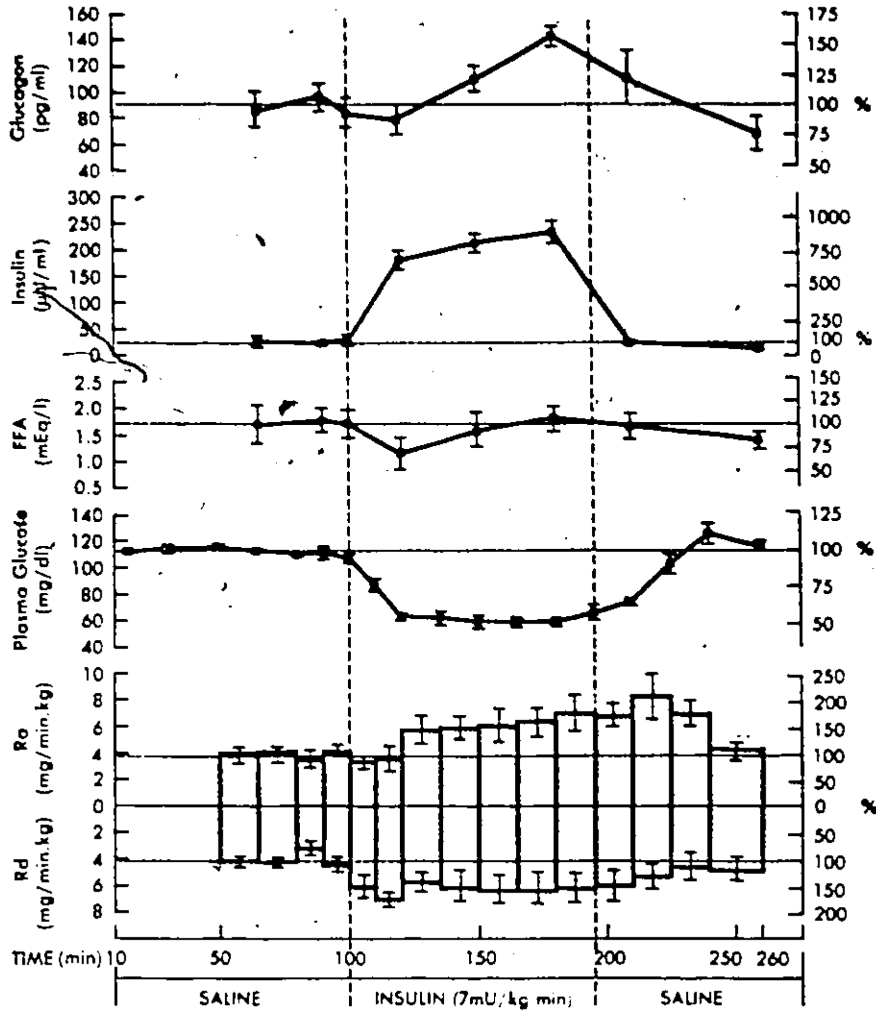


Figure 8

became evident only after t = 120 min. The increase in plasma IRG over the entire period was significant ($F = 9.134$, $p < 0.001$) reaching its maximal level towards the end of the insulin infusion (142 ± 7 $pg.ml^{-1}$, $p < 0.01$). Plasma IRI was raised to about eight times its basal level. The IRI/IRG ratio increased to reach a mximum of 51.3 ± 6.8.

After the cessation of the infusion the concentration of glucose in the plasma returned to its basal level within 45 minutes. There was no change in plasma FFA. Both rates (Ra, Rd) returned to basal by the end of the experiment as did plasma IRI, IRG and the IRI/IRG ratio.

CHAPTER IV

DISCUSSION

The Basal States

The glucose concentration of pancreatectomized and alloxan-diabetic dogs were similar and well above the normal range found in nondiabetic animals. In alloxan-diabetic dogs, hepatic glucose production was significantly higher at a mean value of 5.5 ± 0.4 $mg.min^{-1}.kg^{-1}$. This value is comparable to those published for diabetic dogs (Hetenyi et al., 1980a and Hetenyi et al., 1980b). However, in the pancreatectomized dogs, the Ra was 3.8 ± 0.4 $mg.kg^{-1}.min^{-1}$, only about 30 - 40% above the range found in non-diabetic animals (Hetenyi et al., 1983). This would appear to be inconsistent with the diabetic state. However, the near normal concentration of glucagon in the plasma of pancreatectomized dogs may account for the less elevated Ra. Although hyperglucagonemia has been reported in pancreatectomized dogs (Vranic et al., 1974) it is usually not observable until two days after surgery if insulin treatment is withheld (Matsuyama and Foa, 1974). Our animals were treated with protamine zinc insulin (very long acting), and as indicated by the high level of plasma glucose, the circulating level of insulin at the time of the experiment was inadequate to maintain normoglycemia. Even though insulin therapy

was discontinued for 72 h, insulin was expected to be present in the circulation 24 h before the beginning of the experiment. Its inhibitory effect on gastric glucagon secreting cells (Lefebvre and Luyckx, 1978) was absent for only 24 h, which is not long enough for hyperglucagonemia to develop.

The methylprednisolone treated and the insulin treated diabetic dogs had normal glucose and IRG concentrations. They both had equally elevated plasma insulin levels (22 ± 3 and 18 ± 3 $\mu U.ml^{-1}$ respectively) and similar IRI/IRG ratios as well as similarly elevated FFA concentration, however, the MP-treated animals had a higher Ra than the treated-diabetic dogs. This can be explained by the action of glucocorticoids on gluconeogenesis (Hetenyi et al., 1980b).

The Response of Glucagon Secreting Tissues of Diabetic Dogs to Phlorizin- and Insulin-induced Hypoglycemia.

During the infusion of phlorizin to alloxan-diabetic dogs, the increase in glucagon secretion, in response to rapidly decreasing plasma glucose, did not always occur. However, in pancreatectomized animals such an increase was reproducible, as indicated by the increase of the plasma IRG concentration. The former had an elevated basal glucagon concentration, whereas the latter had a normal IRG concentration before the start of the infusion. In the three alloxan-diabetic dogs that did show some increase in glucagon secretion, the plasma concentration doubled

by the end of the experiment. The response of pancreatectomized dogs was a consistent increase in plasma concentration of approximately 60 $pg.ml^{-1}$. The increase by about 40 to 60 $pg.ml^{-1}$ from a comparable base line was observed in normal, nondiabetic dogs, during similar phlorizin infusions (Gauthier et al.,1980; Gauthier and Hetenyi, 1982 and Gauthier et al., 1983). The infusion of insulin inhibited glucagon release in alloxan-diabetic dogs, confirming observations of Braaten et al. (1974). The plasma IRG remained constant in the pancreatectomized animals, thus insulin prevented the increase of glucagon that was noted during the phlorizin infusion.

In normal dogs the secretion of glucagon is curtailed by insulin secreted into the capillary network within the islet upstream from the alpha-cell (Bonner-Weir and Orci, 1982; Unger, 1983 and Samols et al., 1983). At an inadequately low level of insulin the secretion of glucagon is elevated as it is in alloxan-diabetic dogs. If insulin is infused at a high rate, the high level of insulin in systemic plasma compensates for the lacking intraislet effect and suppresses the secretion of glucagon. Consequently, the concentration of glucagon in plasma decreases. To the contrary, when the concentration of glucose is decreased without insulin, as it is during the infusion of phlorizin, the lessened availability of glucose to the alpha-cell combined with a drop in the already low level of insulin in the plasma will increase glucagon secretion. Therefore, the already high level of

plasma glucagon will increase further. The extent of the response is expected to be correlated to the number of still active beta-cells in the islets, and to the sensitivity of the alpha-cells to hypoglycemia (Table 2). The normal primary effect of an insulin infusion on the alpha cell is to decrease the secretion of glucagon (Samols et al., 1969), whereas the effect of hypoglycemia leads to the release of glucagon. Thus if the subsequent concentration of glucose is sufficiently low, this latter effect prevails over that of insulin (Gauthier et al., 1980). Interestingly when glucose drops to a concentration which are known to stimulate IRG release during an insulin infusion, such a release is not observed in the alloxan-diabetic dog. Therefore we have to assume that the sensitivity of the alpha-cell to hypoglycemia has decreased, while that to insulin has not.

The results presented in this study suggest that gastric alpha-cells are less sensitive to prolonged insulinopenia than the pancreatic alpha-cells since hyperglucagonemia did not appear in the pancreatectomized dogs, whereas it did in the alloxan-diabetic animals. Although insulin has been reported to inhibit gastric glucagon secretion, this was not observed in our experiments on pancreatectomized dogs. Blazquez et al., (1977) reported an inhibitory effect in alloxan-diabetic dogs, when peripheral plasma IRG concentration was in the 500 $pg.ml^{-1}$ range. Pancreatic glucagon secretion was found to be inhibited by insulin only at elevated plasma IRG concentration (greater than 300 $pg.ml^{-1}$)

(Samols et al., 1969 and Samols et al., 1976). Our observations support the hypothesis that this is also true for gastric glucagon secretion although the alpha-cells may be less sensitive to insulin. Lefebvre and Luyckx (1978) reported that at normal IRG concentration, insulin diminished gastric glucagon release during hypoglycemia but did not change the basal glucagon concentration. This is consistent with our observation that the expected increase of glucagon in response to the decreasing plasma glucose was prevented by insulin in the pancreatectomized dogs.

In summary: in diabetic dogs insulin inhibits or prevents high rates of glucagon secretion. Phlorizin-induced moderate hypoglycemia at a high basal glucagon concentration has a variable effect on glucagon secretion, whereas at normal glucagon levels it will consistently promote gastric glucagon secretion in pancreatectomized dogs. Finally, the sensitivity of the pancreatic alpha-cell to hypoglycemia is decreased in alloxan-diabetes.

The Hepatic Response to Phlorizin- and Insulin-induced Hypoglycemia.

The lack of increase in the rate of hepatic glucose production, during phlorizin infusion to alloxan-diabetic dogs, could be accounted for by our finding that neither the plasma concentration of glucagon nor that of epinephrine increased, and that hepatic auto-regulation is lost during prolonged insulinopenia (Davidson et al., 1981). However the increment in

plasma IRG noted in some animals, failed to bring about an increase in glucose production. Stevenson et al. (1983) found in diabetic dogs a decreasing response to measured increments in plasma glucagon, with no further increment once the plasma level of glucagon had reached 400 $pg.ml^{-1}$. Thus, the absence of response can be accounted for by either no glucoregulatory stimulus having been generated, or even if there was, the liver was insensitive to it.

Our observation that an increase in plasma glucagon of approximately 60 $pg.ml^{-1}$ from a normal basal level, which is known to increase Ra by 50 - 80% in normal dogs (Gauthier and Hetenyi, 1982 and Gauthier et al., 1983), does not do so in pancreatectomized dogs, suggests that the insensitivity of the liver to glucagon may not only be related to elevated IRG concentration. It is unclear at this point whether a pre- or post-receptor defect is responsible for the decreased sensitivity, however, the latter is more probable. Epinephrine released during the insulin infusion in amounts similar to those which in normal humans (Gerich et al., 1980; Rizza et al., 1979 and Bolli et al., 1983), increase hepatic glucose production, had no effect on Ra in alloxan-diabetic dogs. Since both glucagon and epinephrine release c-AMP as second messenger, and since insulin treatment restores hepatic sensitivity to glucagon, it is likely that insulin is in some way involved in the maintenance of the response at some post-receptor step.

During the i.v. infusion of insulin Ra was decreased in both alloxan-diabetic and pancreatectomized dogs. This could be explained by the effect of insulin on the liver and the absence of a rise in the concentration of plasma glucagon. The lack of response to the increased epinephrine concentration in the alloxan-diabetic animals was somewhat unexpected, and suggests that the response was inadequate to stimulate the liver, which possibly also has a decreased sensitivity to catecholamines as well as to glucagon. When glucagon secretion is inhibited by somatostatin in humans, epinephrine release is twice as much as in control subjects (without somatostatin) during insulin-induced hypoglycemia, and normoglycemia is restored just as rapidly (Rizza et al., 1979). Also, a 40 fold increase in the concentration of plasma epinephrine was found in normal dogs, during the infusion of insulin, when the concentration of plasma glucose was lowered to between 40 and 50 $mg.dl^{-1}$ (Vranic et al., in press). Since in our experiments the lowest concentration of glucose reached was 69 $\pm$ 18 $mg.dl^{-1}$, direct comparisons of epinephrine secretion rates can not be made safely. However, in our dogs a regulatory response is maintained as indicated by the significant correlation ($r = 0.86$) between the lowest concentration in plasma glucose reached and the concentration of epinephrine in the sample.

Restoration of Non-hypoglycemic Glucose Counterregulation.

A one week long treatment with insulin, sufficient to

maintain normoglycemia in the post-absorptive state, restored the response to phlorizin, and to some extent improved non-hypoglycemic glucose counterregulation in alloxan-diabetic dogs. During the infusion of phlorizin the glucagon concentration increased by approximately 50 pg.ml^{-1}. This increase resulted in a 60 ± 13% increase in the rate of glucose production. In this respect the response to phlorizin was restored (see appendix A). The fact that the glucose concentration in plasma remained constant during the infusion of phlorizin, despite a significant increase in Rd, indicates an increase in sensitivity of the glucoregulatory mechanism, since a 10 - 20% decrease in plasma glucose has been consistently reported in nondiabetic animals infused with phlorizin (Gauthier et al., 1980; Gauthier and Hetenyi, 1982 and Gauthier et al., 1983). It would appear that an increase in the rate of glucose disappearance stimulates glucagon secretion. Thus Rd would be a component of the derivative control of the glucoregulatory mechanism.

The small decrease in plasma IRI observed during the infusion most likely contributed to the rise in the rate of hepatic glucose release. This change in plasma IRI could be the result of the response by the surviving beta-cells to the increased removal of glucose, but especially in view of the lack of any appreciable change in plasma glucose, it is more probably due to a faster metabolism of circulating exogenous insulin.

Such restoration of glucoregulation is not observed in

patients with insulin dependent diabetes mellitus (IDDM) on conventional therapy or treated with continuous subcutaneous insulin infusion (CSII) (Cryer and Gerich, 1983; Unger and Orci, 1981; Unger, 1983 and Bergenstal et al., 1983). Neither was it observed in insulin treated streptozotocin-diabetic rats (Patel, 1983a). A study by Bolli et al. (1983) found that recently diagnosed (less than 12 months) patients under appropriate insulin therapy respond as healthy controls to insulin-induced hypoglycemia, whereas patients with long standing diabetes do not. The glucagon and epinephrine response to hypoglycemia gradually disappeared with duration of diabetes. In diabetic rats, a significant negative correlation was found between the duration of diabetes and the increase in plasma glucagon and epinephrine in insulin-induced hypoglycemia (Patel, 1983b).

Since our dogs were diabetic for 8 to 24 days at the time of the experiment we may only compare our results with those found in recently diagnosed patients. Even then one must be cautious since: (a) a species difference between man and dog is possible and (b) our animals were not exposed to deep hypoglycemia. Also to-date no studies have been published on long standing diabetics exposed to noninsulin-induced moderate hypoglycemia. However, the absence of changes in plasma IRG observed in overt, insulin-induced, hypoglycemia in treated patients (Bolli et al., 1983) indicate that the sensitivity of the alpha-cell to both insulin and hypoglycemia may be curtailed. Thus the impaired glucoregulation

that was observed in alloxan-diabetic dogs, may represent a very early stage of dysfunction, due to beta-cell failure, which is still correctable by the restoration of the normal hormonal milieu. The reported impairment of glucagon secretion in insulin treated rats and humans with long standing diabetes would be a second step: an acquired irreversible defect of the glucoregulatory system involving not only the alpha-cell, since epinephrine release is also impaired (Bolli et al., 1983). This defect may relate to an altered "glucose sensor" rather than to disrupted cell function, since other abnormalities of the diabetic alpha-cell, such as hypersecretion and exaggerated response to arginine, are restored to normal by insulin treatment in long standing diabetics (Gerich et al., 1975).

Is there a Set Point of Plasma Glucose in Diabetes?

The absence of an increase in hepatic glucose production during the phlorizin-induced decrease of plasma glucose concentration indicates, that the elevated level of glycemia in diabetic dogs does not represent the set-point of some regulated system. This is indeed expected, since the increase in glucagon secretion is variable, hepatic autoregulation is abolished and the glucose supply to the brain - the main sensor of overt hypoglycemia - is amply covered by the high level of plasma glucose. This raises the question of the adequacy of glucose supply to the tissues, which in the virtual absence of insulin,

have to use less glucose at a lower level of glycemia. An increased rate of peripheral glucose utilization was shown to lead to an increase in hepatic glucose production in rats (Achou and Hetényi, 1974). This was not observed in our experiments. Since no increase in plasma FFA has been observed during the infusion of phlorizin, we tentatively conclude that internal energy stores of the cell (glycogen, fat) and the already elevated plasma FFA concentration were sufficient to provide fuel for the duration of the experiment.

The Regulatory Response to Hypoglycemic Stimuli in Methylprednisolone Treated Dogs.

The counterregulatory response to a falling concentration of plasma glucose in MP-treated dogs is characterized by an increased sensitivity of the liver to glucagon. This has already been described by Issekutz and Borkow (1972) who found a 20 fold increase in sensitivity to glucagon in MP-treated dogs.The sensitivity to epinephrine and dibutyryl-cyclic AMP was also raised by a factor of 12 (Issekutz and Borkow, 1973).

In our experiments the increment in Ra during the phlorizin-induced increase in the rate of glucose disappearance was of the same magnitude but occured at a much lower level of plasma glucagon than in untreated animals: in comparison to a maximum 2 fold increase in plasma glucagon in normal animals, the maximum increment was only 24 ± 10% (19 ± 9 $pg.ml^{-1}$). In all the

experiments the concentration of IRG at the end of the infusion period was higher than basal level. Because of interanimal variations and in view of the limits of the precision of the radioimmunoassay, the increment, although consistent, was not statisticaly significant. Nevertheless this small increase in plasma IRG was sufficient to raise Ra and restore normoglycemia within 20 minutes. This indicates that it is the change in concentration of plasma glucose, however small or transient it may be, that regulates the release of glucagon. Once normoglycemia has been restored, the stimulus for an increased secretion of glucagon is expected to disappear temporarily, to be reactivated by the next small drop in plasma glucose, producing another small burst of glucagon release. Thus the heightened sensitivity of the liver to glucagon creates a sensitive feedback loop operating between plasma glucose and IRG concentration. With the experimental design used, taking glucagon samples every 30 min and averaging Ra over periods of 15 min, the expected oscillation can not be shown, particularly not if they are of different frequencies in different dogs.

The heightened sensitivity of the regulatory response to hypoglycemia is evidenced also by the rapid rate of recovery. The return of plasma glucose to the basal level, from about the same low level of glucose of 40 to 50 $mg.dl^{-1}$, took about 100 min in the normal dogs (Gauthier et al., 1980), whereas in MP-treated animals a significant overshoot was observed 45 min after the

infusion of insulin was discontinued (Figure 8).

The way MP treatement enhances the effect of glucagon is not completely understood. In adrenalectomized rats glucagon is shown not to stimulate gluconeogenesis, although it raises the concentration of c-AMP in the liver (Newsholme and Start, 1973). Treatement with dexamethasone restores the gluconeogenic activity of glucagon. It was concluded that corticosteroids influence the reaction between gluconeogenic enzymes and c-AMP. In rat hepatocytes the activity of PEPCK was stimulated by glucagon and could be increased further synergistically by cortisol (Schudt et al., 1980).

Furthermore, the activity of the glycogenolytic enzyme phosphorylase kinase as well as the concentration of phosphorylase were found to be decreased in hepatocytes of adrenalectomized rats (Chan et al., 1979 and Schaeffer et al., 1969). In these animals neither epinephrine nor c-AMP raised the level of glucose in the plasma. Treatment with hydrocortisone restored both the normal level of hepatic phosphorylase and the glycemic response. It is thus conceivable that treatment with methylprednisolone increases the synthesis and activity of phosphorylase and phosphorylase kinase, and in this way potentiates the effect of glucagon on glycogenolysis, an explanation favoured by Issekutz and Borkow (1972).

Another potential factor in the mechanism of increased glucagon sensitivity is the resistance to insulin in

hypercorticism evident both at the level of the liver and the periphery (Rizza et al., 1982). The insulin resistance is due to a post-receptor defect. Thus it is likely that an increase in plasma IRG in MP-treated dogs will have a larger effect than in normal dogs since the antagonistic effect of insulin is diminished.

Comparison Between Methylprednisolone Treated and Diabetic Dogs.

In methylprednisolone treated dogs both Ra and gluconeogenesis are at a rate much above normal (Ninomiya et al., 1964 , Issekutz and Borkow, 1972 and Hetenyi et al., 1980). Both rates are at levels observed in uncontrolled diabetes. The difference in the response to phlorizin proves that a high Ra is not in itself a limiting factor for the glucoregulatory response since Ra was increased when MP-treated animals were exposed to moderate as well as to deep hypoglycemic stimuli.

Hepatic glucose production is the result of both glycogenolysis and gluconeogenesis, and a counterregulatory response consists of a rapid transient increase in the rate of glycogen breakdown followed by a slow increase in the rate of glucose synthesis (Cherrington et al., 1981 and Gauthier and Hetenyi, 1982). Nevertheless, the MP-treated and the alloxan-diabetic dog differ in two major ways with respect to glucose output. First, the concentration of glycogen in the liver cell of MP-treated animals is considerably higher than in the diabetic dogs. Thus MP-treated dogs have a large store of rapidly mobilizable glucose, whereas diabetic dogs have not. Secondly, the

fraction of glucose output due to gluconeogenesis, as estimated by the incorporation of ^{14}C-atoms from labelled alanine into glucose, is by 53% larger in MP-treated than in diabetic dogs. This is due to an increased delivery of substrate to the liver as opposed to diabetes where a larger fraction of substrate arriving at the liver is turned into glucose (Hetenyi et al., 1980b). The fact that the elevated rate of gluconeogenesis in MP-treated and diabetic dogs is due to different mechanisms (substrate supply vs enzyme activation), and that glycogen reserves also differ, explains why the latter can not rapidly counterregulate in response to decreasing glycemia while the former can, even if they both have similarly elevated rates of glucose production.

Surprisingly both MP-treated and the insulin treated-diabetic animals showed a highly sensitive counterregulatory response. Both had an elevated IRI/IRG ratio with high insulin and normal glucagon. Both maintained a constant glucose concentration near to the basal level during most of the phlorizin infusion in spite of a difference in their basal rate of glucose production and hepatic sensitivity to glucagon: in diabetic animals the peripheral glucagon concentration rose higher to achieve the same effect on Ra. However, both animals responded equally quickly to the increased rate of glucose removal. It is possible that the elevated IRI concentration in the treated-diabetic dog may favour a higher than normal rate of glycogenesis by maintaining the activity of glucokinase and glycogen synthetase (Katz et al., 1979 and Davidson et al., 1981). Thus, as in the case of the MP-treated

dog the hepatocyte glycogen concentration would be elevated, permitting a rapid response to glycogenolytic stimuli.

Summary

The results of these experiments revealed that insulin deprived diabetic dogs do not raise the rate of hepatic glucose production when exposed to insulin-induced hypoglycemia or to a phlorizin-induced decrease in the concentration of plasma glucose. In the case of insulin-induced hypoglycemia this is due to both the inhibitory effect of insulin on glucagon secretion and its direct effect on hepatic glucose production. During the infusion of phlorizin, the absence of a regulatory response is due to the non-systematic increase of glucagon release and to the insensitivity of the diabetic liver to glucagon. The former is associated with a disrupted intraislet hormonal milieu, and the latter may be the result of hyperglucagonemia and/or prolonged insulinopenia. The glucoregulatory response can be restored by adequate insulin therapy. The high rate of glucose production with an elevated gluconeogenic flux does not, in itself, curtail a further increase in Ra in response to decreasing glycemia. The increased sensitivity of the glucoregulatory mechanism of the MP-treated animals is related on one hand to the increased hepatic sensitivity to glucagon, and on the other hand, as in the insulin treated diabetic dogs, to an increased rate of glycogen synthesis in the liver, due to elevated plasma insulin concentration and IRI/IRG ratio.

References

Achou, C.J. and Hetenyi, G., Jr. (1974). Is the peripheral utilization of glucose a regulator of hepatic glucose production and glycemia? Can. J. Physiol. Pharmcol 52: 158-165.

Alberti, K.G.M.M., Christensen, N.J., Christensen, S.E., Hansen, A.P., Iversen, J., Lundboek, K., Seyer-Hansen, K. and Orskov, H. (1973). Inhibition of insulin secretion by somatostatin. Lancet 2: 1299-1301.

Altman, P.L. (1961). Blood and other body fluids. Biological Handbooks, D.S. Dittmer, Editor, Washington, D.C. Federation of American Societies for Experimental Biology.

Ashcroft, S.J.H. (1981). Metabolic control of insulin secretion. In. The Islets of Langerhans, S.J. Cooperstein and D. Watkins, editors, New York: Academic Press Inc., pp. 117-148.

Assimacopoulos-Jeannet, F.D., Blackmore, P.F. and Exton, J.H. (1977). Studies on α-adrenergic activation of hepatic glucose output: Studies on role of calcium in α-adrenergic activation of phosphorylase. J. Biol. Chem. 252: 2662-2669.

Bailey, C.C. and Hagan, W.H. (1944). Alloxan diabetes in rabbit; consideration of morphologic and physiologic changes. Am. J. Med. Sci. 208: 450-461.

Baxter, J.D. and Forsham, P.H. (1972). Tissue effects of glucocorticoids. Am. J. Med. 53: 573-

Benson, J.W., Johnson, D.G., Palmer, J.P., Werner, P.L. and Ensinck, J.W. (1977). Glucagon and catecholamine secretion during hypoglycemia in normal and diabetic man. J. Clin. Endocrinol. Metab. 44: 459-464

Bergenstal, R.M., Polorsky, K.S., Pons, G., Jaspan, J.B. and Rubenstein, A.H. (1983). Lack of glucagon response to hypoglycemia in Type I diabetics after long-term optimal therapy with a continuous subcutaneous insulin infusion pump. Diabetes 32: 398-402.

Bishop, J.S., Steele, R., Altszuler, N.A., Rathgeb, I., Bjerknes, C. and DeBodo, R.C. (1967). Diminished responsiveness to insulin in growth hormone treated normal dog. Am. J. Physiol. 212: 272-278.

Blazquez, E., Munoz-Barragan, L, Patton, G.S., Dobbs, R.E. and Unger, R.H. (1977). Demonstratjons of gastric glucagon hypersecretion in insulin-deprived alloxan-diabetic dogs. J. Lab. Clin. Med. 89: 971-977.

Bloom, S.R., Barnes, A.J., Bryant, M.G. and Alberti, K.G.M.M. (1976). Plasma glucagon in diabetes resulting from total pancreatectomy. Metabolism 25: 1481-1482.

Bolli, G., DeFeo, P., Compagnucci, P., Cartechini, M.G., Angeletti, G., Santeusanio, F., Brunetti, P. and Gerich, J.E. (1983). Abnormal glucose counterregulation in insulin-dependent diabetes mellitus: Interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. Diabetes 32: 134-141.

Bonner-Weir, S. and Orci, L. (1982). New perspectives on the microvasculature of the islets of Langerhans in the rat. Diabetes 31: 883-889.

Boquist, L., Lorentzon, R. (1980). Study of the early blood glucose response of mice to alloxan. Diabetes Metab. 6: 55-58.

Braaten, J.T., Faloona, G.R. and Unger, R.H. (1974). The effect of insulin on the alpha-cell response to hyperglycemia in long-standing alloxan diabetes. J. Clin. Invest. 53: 1017-1021

Brekke, I.B., Oyasoeter, S, Vidnes, J. (1982). Long-term effect of pancreas transplantation on diabetic hyperglucagonemia. Eur. Surg. Res. 14: 211-220.

Bucolo, R.J., Bergman, R.N., Marsh, D.J. and Yates, F.E. (1974). Dynamics of glucose autoregulation in the isolated, blood perfused canine liver. Am. J. Physiol. 227: 209-217.

Campbell, J. and Rastogi, K.S. (1967). Elevation in serum insulin, albumin and FFA, with gains in liver lipid and protein, induced by glucocorticoid treatment in dogs. Can. J. Physiol. Pharmacol. 46: 421-429.

Campfield, A.L. (1983). Mathematical analysis of competitive protein binding assay. In. Principles of Competitive Protein-Binding Assays, 2nd Edition, W.D. Odell and P. Franchimont, editors, New York, John Wiley & Son Inc, pp. 125-148.

Carpentier, J.-L., Malaisse-Lagae, F., Muller, W.A. and Orci, L. (1977). Glucagon release from pancreatic islets: A combined morphological and functional approach. J. Clin. Invest. 60: 1174-1182.

Chan, T.M., Steiner, K.E., Exton, J.H. (1979). Effects of adrenalectomy on hormone action on hepatic glucose metabolism: Impaired glucagon activation of glycogen phosphorylase in hepatocytes from adrenalectomized rats. J. Biol. Chem. 254: 11374-11378.

Charles, M.M., Lawecki, J., Pictet, R. and Grodsky, G.M. (1975). Insulin secretion: Interrelationship of glucose cyclic adenosine 3':5'-Monophosphate and calcium. J. Biol. Chem. 250: 6134-6140.

Cherrington, A.D., Williams, P.E., Abon-Manrad, N., Lacy, W.W., Steiner, K.E. and Lilijenquist, J.E. (1982). Insulin as a mediator of hepatic glucose uptake in the conscious dog. Am. J. Physiol. 242: E97-E101.

Cherrington, A.D., Williams, P.E., Shulman, G.I. and Lacy, W.W. (1981). Differential time course of glucagon's effect on glycogenolysis and gluconeogenesis in the conscious dog. Diabetes 20: 180-187.

Clutter, W.E., Bier, D.M. Shah, S.D. and Cryer, P.E. (1980). Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. J. Clin. Invest. 66: 94-101.

Colton, T. (1974). Inference on means. In. Statistics in Medicine. Boston, MA, Little, Brown and Company, pp. 99-150.

Cooperstein, S.J. and Watkins, D. (1981). Action of toxic drugs on islet cells. In. The Islets of Langerhans, S.J. Cooperstein and D. Watkins, editors, New York, Academic Press Inc., pp. 387-425.

Cowan, J.S. and Hetenyi, G., Jr. (1971). Glucoregulation responses in normal and diabetic dogs recorded by a new tracer method. Metabolism 20: 360-372.

Cryer, P.E. (1981). Glucose counterregulation in man. Diabetes 30: 261-264.

Cryer, P.E. and Gerich, J.E. (1983). Relevence of glucose counter-regulatory systems to patients with diabetes: critical roles of glucagon and epinephrine. Diabetes Care 6: 95-99.

Curry, D.L., Bennet, L.L. and Grodsky, G.M. (1968). Dynamics of insulin secretion by perfused rat pancreas. Endocrinology 83: 572-584.

Czech, M.P. (1980). Insulin action and the regulation of hexose transport. Diabetes 29: 399-409.

Czech, M.P. (1981). Insulin Action. Am. J. Med. 70: 142-150.

Davidson, M.B. (1981). Autoregulation by glucose of hepatic glucose balance: Permissive effect of insulin. Metabolism 30: 279-284.

DeBodo, R.C., Steele, R., Altszuler, N., Dunn, A. and Bishop, J. (1963). On the hormonal regulation of carbohydrate metabolism. Recent Prog. Horm. Res. 19: 445-488.

Dixit, P.K., Lowe, I. and Lazarow, A. (1962). Effect of alloxan on insulin content of micro-dissected mammalian pancreatic islets. Nature 195: 388-389.

Dupre, J., Ross, S.A., Waston, D. and Brown, J.C. (1973). Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J. Clin. Endocrinol. Metab. 37: 820-828.

Drost, H., Grüneklee, D., Kley, H.K., Wiegelmann, W., Krusbemper, H.L. and Gries, F.A. (1980). Untersuchungen zur glukagon-, STH- und cortisolsekretion bei insulininduzierter hypoglykamiebei insulinabhangigen diabetikern (JDD) ohne autonome neuropathie. Klin. Wochenschr 58: 1197-1205.

Dunn, J.S., Duffy, E., Gilmour, M.K., Kirpatrick, J. and McLetchie, N.G.B. (1944). Further observations on the effects of alloxan on the pancreatic islets. J. Physiol. 103: 233-243.

Dunnett, W. (1955). A multiple comparison procedure for comparing several treatments with a control. Journal of the American Statistical Association 50: 1096-1121.

Eigler, N., Sacca, L. and Sherwin, R.S. (1979). Synergistic interaction of physiologic increments of glucagon, epinephrine, and cortisol in the dog. J. Clin. Invest. 63: 114-123.

Exton, J.H. and C.R. Park (1969). Control of gluconeogenesis in liver III. Effects of L-lactate, pyruvate, fructose, glucagon, epinephrine and adenosine 3',5' monophosphate on gluconeogenic intermiates in the perfused rat liver. J. Biol. Chem. 244: 1424-1433.

Faloona, G.R. and Unger, R.H. (1974). Glucagon. In. Methods of Hormone Radioimmunoassay, B.M. Jaffe and H.R. Berman, editors, New York, Academic Press, pp. 317-330.

Ganong, W.F. (1979). Neurophysiologic basis of instinctual behavior and emotions. In. Review of Medical Physiology, 9th ed., Los Altos, California, Lange Medical Publications, pp. 182-196.

Gauthier, C. and Hetenyi, G., Jr. (1982). Origin of glucose released in the regulatory response against hypoglycemia. Metabolism 31: 147-153.

Gauthier, C., Vranic, M. and Hetenyi, G., Jr. (1980). Importance of glucagon in regulatory rather than emergency responses to hypoglycemia. Am. J. Physiol. 238: E131-E140.

Gauthier, C., Vranic, M. and Hetenyi, G., Jr. (1983). Nonhypoglycemic glucoregulation: Role of glyceral and glucoregulatory hormones. Am. J. Physiol. 244: E373-E379.

Gerich, J., Cryer, P. and Rizza, R. (1980). Hormonal Mechanisms in acute glucose counterregulation: The relative roles of glucagon, epinephrine, norepinephrine, growth hormone, and cortisol. Metabolism 29: 1164-1175.

Gerich, J.E., Lorenzi, M., Tsalkian, E. and Karam, J.H. (1976). Studies on the mechanism of epinephrine-induced hyperglycemia in man. Evidence for participation of pancreatic glucagon secretion. Diabetes 25: 65-71.

Gerich, J.E., Tsalikian, E., Lorenzi, M., Schneider, V., Bohannon, N.V., Gustafson, G. and Karam, J. (1975). Normalization of gastric hyperglucagonemia excessive glucagon response to intravenous arginine in human diabetes mellitus by prolonged infusion of insulin. J. Clin. Endocrinol. Metab. 41: 1178-1179

Goresky, C.A. and Nadeau, B.E. (1974). Uptake of materials by the intact liver. The exchange of glucose across the cell membranes. J. Clin. Invest. 53: 635-645.

Goto, Y., Seino, Y., Tomohiko, T., Inone, Y., Kadowaki, S., Mori, K. and Imura, H. (1978). Modulation by alloxan of glucagon and insulin secretion in the isolated perfused rat pancreas. Endocrinology 102: 1496-1500.

Grankvist, K., Lernmark, A.L. and Taljedal, I.B. (1977). Alloxan cytotoxicity in ultra:microscopic phatometric analyses of trypan blue uptake by pancreatic islet cells in suspension. Biochem. J. 162: 19-24.

Granner, D., Andreone, T., Saski, K. and Beale, E. (1983). Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. Nature 305: 549-551.

Grodsky, G.M. (1979). Chemistry and functions of the hormones: I. Thyroid, pancreas, adrenal and gastrointestinal tract. In. Review of Physiological Chemistry, (7th Edition), H.A. Harper V.W. Rodwell and P.A. Mayes, Editors, Los Altos, California, Lange Medical Publications, pp. 511-555.

Hahn, H.-J., Gylfe, E. and Hellman, B. (1980). Calcium and pancreatic β-cell function. 7 Evidence for cyclic AMP-induced translocation of intracellular calcium. Biochim. Biophys. Acta 630: 425-432.

Hanson, R.W., Fisher, L., Ballard, F.J. and Reshef, L. (1973). The regulation of phosphenolpyruvate carboxykinase in fetal rat liver. Enzyme 15: 97-110.

Heding, L.G. (1971). Radioimmunological determination of pancreatic and gut glucagon in plasma. Diabetologia 7: 10-19.

Hepp, D., Challoner, D.R. and Williams, R.H. (1968). Studies on the action of insulin in isolated adipose tissue cells. I. Stimulation of incorperation of ^{32}P-labelled inorganic phosphate into mononucleotides in the absence of glucose. J. Biol. Chem. 243: 4020-4026.

Herbert, W., Lau, K.S., Gottlieb, C.W. and Bleicher, S. (1965). Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. 25: 1375-1384.

Hers, H.G., DeWulf, H., Stalmans, W. and Van Den Berghe, G. (1970). The control of glycogen synthesis in the liver. Adv. Enzyme Regul. 8: 171-190.

Hetenyi, Jr., G. (1979). Correction factor for the estimation of plasma glucose synthesis from the transfer of ^{14}C-atoms from labelled substrate in vivo: A preliminary report. Can. J. Physiol. Pharmacol. 57: 767-770

Hetenyi, G., Perez, G. and Vranic, M. (1983). Turnover and precursor-product relationships of nonlipid metabolites. Physiol. Rev. 63: 606-667.

Hetenyi, Jr., G., Layberry, R.A., Forter, D.M. and Berman, M. (1980a). Transfer of carbon atoms among circulating glucose, alanine and lactate in pancreatectomized dogs. Am. J. Physiol. 239: E39-E44.

Hetenyi, Jr., G., Pagurek, B., Dittmar, E.A. and Ferrarotto, C. (1980b). The effects of methylprednisalone and diabetes on the turnover of alanine and on the transfer of carbon atoms from alanine to pyruvate and glucose in dogs. Can. J. Physiol. Pharmacol. 58: 787-796.

Hilsted, J., Madsbad, S., Krarup, T., Sestoft, L., Christensen, N.J., Tornier, B. and Gabbo, H. (1981). Hormonal, metabolic and cardiovascular responses to hypoglycemia in diabetic autonomic neuropathy. Diabetes 30: 626-633.

Itoh, M., Reach, G., Furman, B. and Gerich, J. (1981). Secretion of glucagon. In. The Islets of Langerhans, S.J. Cooperstein and D. Watkins, editors, New York: Academic Press Inc., pp. 117-148.

Issekutz, Jr., B. and Borkow, I. (1972). Effect of catecholamines and dibutyryl-cyclic-AMP on glucose turnover, plasma FFA and insulin in dogs treated with methylprednisolone. Can. J. Physiol. Pharmacol. 50: 999-1006.

Issekutz, Jr., B. and Borkow, I. (1973). Effect of glucagon and glucose load on glucose kinetics, plasma FFA and insulin in dogs treated with methyl-prednisolone. Metabolism 22: 39-49.

Issekutz, B., Issekutz, T., Elahi, D. and Borkow, I. (1974). Effect of insulin infusion on the glucose kinetics in alloxan-streptozotocin diabetic dogs. Diabetologia 10: 323-328.

Iynedjian, P.B. and Hanson, R.W. (1977). Increase in level of functional messenger RNA coding for phosphoenalpyruvate carboxykinase (GTP) during induction by cyclic adenosine 3':5'-monophosphate. J. Biol. Chem. 252: 655-662.

Iynedjian, P.B., Kioussis, D., Ruiz, J.P.G. and Hanson, R.W. (1978). Hormonal regulation of phosphenal pyruvate carboxykinase synthesis. In. Regulatory Mechanisms in Carbohydrate Metabolism, V. Essman, editor, Oxford: Pergamon Press, pp. 83-92.

Jain, K., Logothetopoulos, J. and Zucker, P. (1975). The effects of D and L-glyceraldehyde on glucose oxidation, insulin secretion and insulin biosynthesis by pancreatic islet of the rat. Biochim. Biophys. Acta 399: 384-394.

Jensen, D. (1980). The pituitary gland. In. The Principles of Physiology, 2nd Edition. New York Appleton-Century-Crofts.

Jungas, R.L. (1975). Metabolic effects on adipose tissue in vitro. In. Insulin II., B. Hellman and F.V. Brchhansen, editors, Handb. Exp. Pharm. 32: 371-412.

Kadish, A.H., Little, R.L. and Sternberg, J.C. (1968). A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. Clin. Chem. 14: 116-131.

Katz, N.R., Wanck, M.A. and Wilson, P.T. (1979). Induction of glucokinase by insulin under the permissive action of dexamethasone in primary rat hepatocyte culture. Biochem. Biophys. Res. Commun. 88: 23-29.

Kennedy, W.B. and Lukens, F.D. (1944). Observations on alloxan diabetes. Proc. Soc. Exp. Biol. Med. 57: 143-149.

Keppel, G. (1973). Multiple comparisons. In. Design and Analysis: A Researcher's Handbook, Englewood Cliffs, N.J., Prentice-Hall Inc., pp. 133-163 and pp. 244-246.

Kleinbaum, J. and Shamoon, H. (1983). Impaired counterregulation of hypoglycemia in insulin dependent diabetes mellitus. Diabetes 32: 493-498.

Koerker, D., J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C.J., Ensinck, J. and Gale, C.C. (1974). Somatostatin: Hyrothalamic inhibitor of the endocrine pancreas. Science 184: 482-484.

Kolodny, E.H., Kline, R. and Altszuler, N. (1962). Effect of phlorizin on hepatic glucose output. Am. J. Physiol. 202: 149-154.

Larner, J., Galasko, G., Cheng, K., Depaoli-Roach, A.A., Huang, L., Daggy, P. and Kellogg, J. (1979). Generation by insulin of a chemical mediator that controls protein phospharylation and Dephosphorylation. Science 206: 1408-1410.

Laurell, S. and Tibbling, G. (1966). Calorimetric micro-determination of free fatty acids in plasma. Clin. Chim. Acta 16: 57-62.

Lazarus, S.S., Barden, H. and Bradshaw, M. (1962). Pancreatic beta cells and alloxan toxicity-enzymatic histochemistry and toxic mechanism. Arch. Pathol. 73: 210-222.

Lefebvre, P.J. and Luyckx, A.S. (1978). Glucose and insulin in the regulation of glucagon release from the isolated perfused dog stomach. Endocrinology 103: 1579-1582.

Lehninger, A.L. (1975). Glycolysis. In. Biochemistry, New York, Worth Publisher, Inc., pp. 417-476.

Lehninger, A.L. (1975). The biosynthesis of carbohydrates. In. Biochemistry, New York, Worth Publisher Inc., pp. 623-658.

Liljenquist, J.E., Muller, G.L., Cherrington, A.D., Perry, J.M. and Rabinowitz, D. (1979). Hyperglycemia per se (insulin and glucagon withdrawn) can inhibit hepatic glucose production in man. J. Clin. Endocrinol. Metab. 48: 171-175.

Lukens, F.D.W. (1948). Alloxan diabetes. Physiol. Rev. 28: 304-330.

Lunquist, I., Rerup, C.C. (1967). On the development of alloxan diabetes in mice. Eur. J. Pharmacol. 2: 35-41.

MacGorman, L. and Rizza, R. (1980). Insulin-like and insulin antagonistic effects of growth hormone on glucose production and clearance in man. (Abstract). Diabetes 29, Suppl. 2: 56A.

Massagne, J., Pilch, P.F. and Czech, M.P. (1980). Electrophoretic resoltuion of three major insulin receptor structures with unique subunit stoichiometries. Proc. Natl. Acad. Sci. U.S.A. 77: 7137-7141.

Matsuyama, T. and Foa, P.P. (1974). Plasma glucose, insulin, pancreatic and enteroglucagon levels in normal and depancreatized dogs. Proc. Soc. Exp. Biol. Med. 147: 97-102.

Markowitz, M.B.E., Archibald, J. and Downie, H.G. (1959). Experimental surgery of the pancreas. In. Experimental surgery. Baltimore, The Williams and Wilkins Company, pp. 351-355.

Mayes, P.A. (1979). Metabolism of carbohydrates. In. Review of Physiological Chemistry, H.A. Harper, V.W. Rodwell and P.A. Mayes, editors, Los Altos, Lange Medical Publication, pp. 294-320.

Meissner, H.P. and Preissler, M. (1979). Glucose induced changes of the membrane potential of pancreatic β-cell: Their significance for the regulation of insulin release. Adv. Exp. Med. Biol. 119: 97-107.

Merimee, T.J., Rakin, D. (1973). A survey a growth hormone secretion and action. Metabolism 22: 1235-1251.

Montague, W. (1983a). Glucagon. In. Diabetes and the Endocrine Pancreas, New York: Oxford University Press Inc., pp. 86-102.

Muller, W.A., Girardier, L., Seydoux, J., Berger, M., Renold, A.E. and Vranic, M. (1978). Extrapancreatic glucagon and glucagon-like immunoreactivity in depancreatized dogs. J. Clin. Invest. 62: 124-132.

McDaniel, M.L. and Lacy, P.E. (1981). Interaction of cell organells in insulin secretion. In. The Islets of Langerhans, S.J. Cooperstein and D. Watkins, editors, New York: Academic Press Inc., pp. 97-115.

McLetchie, N.G.B. (1982). Alloxan: The sorcerer and his apprenctice. Diabetologia 23: 72-73.

Nash, Jr., T.P. (1927). Phlorizin diabetes. Physiol. Rev. 7: 385-430.

Newsholme, E.A. and Start, C. (1973). Regulation of carbohydrate metabolism in liver. In. Regulation in Metabolism, London, John Wiley and Sons, pp. 247-292.

Ninomiya, R., Forbath, N.F. and Hetenyi, Jr., G. (1965). Effects of adrenal steroids on glucose kinetics in normal and diabetic dogs. Diabetes 14: 729-739.

Noe, B.D., Fletcher, D.J. and Bauer, G.E. (1981). Biosynthesis of glucagon and somatostatin. In. The Islets of Langerhans. S.J. Cooperstein and D. Watkins, Editors, New York, Academic Press Inc., pp. 189-255.

Olefsky, J.M. (1981). Insulin resistance and insulin action, an in vitro and in vivo perspective. Diabetes 30: 148-162.

O'Malley, B.W., Schwartz, R.J. and Schrader, W.T. (1976). A review of regulation of gene expression by steroid hormone receptors. J. Steroid Biochem. 7: 1151-1159.

Orci, L., Amherdt, M., Malaisse-Lagae, F., Ravazzola, M., Malaisse, W.J., Perrelet, A. and Renold, A.E. (1976a). Islet cell membrane alteration by diabetogenic drugs. Lab. Invest. 34: 451-454.

Orci, L., Amherdt, M., Stauffacher, W., Like, A.A., Rouiller, C. and Renold, A.E. (1972). Structural changes in membranes of beta cells exposed to alloxan and streptozotocin demonstrated by freeze-etching. Diabetes 21: 326.

Orci, L., Bordi, C., Unger, R.H. and Perrelet, V.A. (1983). Glucagon- and glicentim-producing cells. In. Glucagon I-H and Book of Experimental Pharmacology, Vol. 66/I, P.J. Lefebvre, Editor, Springer Verlag, Berlin, pp. 57-79.

Orci, L., Like, A.A., Amherdt, M., Blondel, B., Kanazawa, Y., Marliss, E.R., Lambert, A.E., Wollheim, C.B. and Renold, A.E. (1973). Monolayer cell culture of neonatal rat pancreas: An ultrastructural and biochemical study of functioning endocrine cells. J. Ultrastruct. Res. 43: 270-297.

Orly, J. and Schramm, M. (1976). Coupling of catecholamine receptor from one cell with adenylate cyclase from another cell by cell infusion. Proc. Natl. Acad. Sci. U.S.A. 73: 4410-4414.

Pagliara, A.S., Stilling, S.N., Zawalich, W.S., Williams, A.D. and Matschinsky, F.M. (1977). Glucose and 3-O-methylglucose protection against alloxan poisoning of pancreatic alpha and beta cells. Diabetes 26: 973-979.

Patel, D.G. (1983a). Effect of prolonged insulin treatment on blunted plasma catecholamine and glucagon increase during insulin hypoglycemia in streptozotocin diabetic rats. Metabolism 32: 377-382.

Patel, D.G. (1983b). Lack of glucagon response to hypoglycemia in long-term experimental diabetic rats. Diabetes 32: 55-60.

Permutt, M.A. (1981). Biosynthesis of insulin. In. The Islets of Langerhands, S.J. Cooperstein and D. Watkins, editors, New York, Academic Press Inc., pp. 75-95.

Pilkis, S.J., Park, C.R. and Claus, T.H. (1978). Hormonal control of hepatic gluconeogenesis. Vit. Horm. 36: 383-460.

Radziuk, J., Norwich, K.H. and Vranic, M. (1978). Experimental validation of measurements of glucose turnover in nonsteady state. Am. J. Physiol. 234: E84-E93.

Rerup, C.C. (1970). Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol. Rev. 22: 485-518.

Riou, J.P., Claus, T.H. and Pilkis, S.J. (1976). Control of pyruvate kinase activity by glucagon in isolated hepatocytes. Biochem. Biophys. Res. Commun. 73: 591-599.

Riou, J.P., Claus, T.H. and Pilkis, S.J. (1978). Stimulation by glucagon of in vivo phosphorylation of rat hepatic pyruvate kinase. J. Biol. Chem. 253: 656-659.

Rizza, R.A., Cryer, P.E. and Gerich, J.E. (1979). Catecholamine and growth hormone in human glucose counterregulation. Effects of somatostatin and combined α- and β-adrenergic blockade on plasma glucose recovery and glucose flux rates after insulin-induced hypoglycemia. J. Clin. Invest. 64: 62-71.

Rizza, R.A., Mandarino, L.J. and Gerich, J.E. (1982). Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a post receptor defect of insulin action. J. Clin. Endocrinol. Metab. 54: 131-138.

Rodbell, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 284: 17-22.

Roscoe, J.T. (1975). Multiple comparisons in the analysis of variance. In. Fundamental Research Statistics for the Behavioral Sciences, 2nd Edition, New York, Holt, Rinehard and Winston Inc., pp. 309-322.

Ruiz, J.P.G., Ingram, R. and Hanson, R.W. (1978). Changes in hepatic messenger RNA for phosphoenolpyruvate carboxykinase (GTP) during development. Proc. Natl. Acad. Sci. U.S.A. 75: 4189-4193.

Sacca, L., Sherwin, R., Hendler, R. and Felig, P. (1979). Influence of continuous physiologic hyperinsulinemia on glucose kinetics and conterregulatory hormones in normal and diabetic humans. J. Clin. Invest. 63: 849-857.

Samols, E. and Harrison, J. (1976). Intraislet negative insulin-glucagon feedback. Metabolism 25: 1443-1447.

Samols, E., Marri, G. and Marks, V. (1965). Promotion of insulin secretion by glucagon. Lancet 2: 415-416.

Samols, E., Tyler, J.M. and Mialhe, P. (1969). Suppression of pancreatic glucagon release by the hypoglycaemic sulphonylureas. Lancet 1: 174-176.

Samols, E., Weir, G.C. and Bonner-Weir, S. (1983). Intraislet insulin-glucagon somatostatin relationships. In. Glucagon II - Handbook of Experimental Pharmacology, Vol. 66/II, P.J. Lefebvre, editor, Springer-Verlag, Berlin, pp. 133-173.

Santiago, J.V., Clarke, W.L., Shah, S.D. and Cryer, P.E. (1980). Epinephrine, glucagon and growth hormone release in association with physiological decrements in the plasma glucose concentration in normal and diabetic man. J. Clin. Endocrinol. Metab. 51: 877-883.

Schaeffer, L.D., Chenoweth, M. and Dunn, A. (1969). Adrenal corticosteroid involvement in the control of liver glycogen phosphorylase activity. Biochim. Biophys. Acta 192: 292-300.

Schudt, C. (1980). Regulation of phosphoenalpyruvate carboxykinase by glucagon and glucocorticoids in primary cultures of rat hepatocytes. Biochim. Biophys. Acta 628: 277-285.

Seals, J.R. and Czech, M.P. (1980). Evidence that insulin activates an intrinsic plasma membrane protease in generating a secondary chemical mediator. J. Biol. Chem. 255: 6529-6531.

Shulman, G.I., Liljenquist, J.E. and Williams, P.E. (1978). Glucose disposal during insulinopemia in somatostatin treated dogs. J. Clin. Invest. 62: 487-491.

Silverman, M., Aganon, M.A. and Chinard, F.P. (1970). D-glucose interactions with renal tubule cell surfaces. Am. J. Physiol. 218: 735-742.

Silverman, M. and Black, J. (1975). High affinity phlorizin receptor sites and their relation to the glucose transport mechanism in the proximal tubule of dog kidney. Biochim. Biophys. Acta 394: 10-30.

Sloan, I.G., Sawh, P.C. and Bihler, I. (1978). Influence of adrenaline on sugar transport in soleus and red skeletal muscle. Mol. Cell. Endocrinol. 10: 3-12.

Snedecor, G.W. (1956). Two-way experiments. Analysis of variance. In. Statistical Methods, Ames, IA; Iowa State Univ. Press, pp. 291-328.

Sole, M.J. and Hussain, M.N. (1977). A simple specific radioenzymatic assay for the simultaneous measurement of picogram quantities of norepinephrine, epinephrine and dopamine in plasma and tissue. Biochem. Med. 18: 301-307.

Soling, H.D. and Seufert, C.D. (1975). Direct in vivo effects of insulin on liver metabolism. In. Insulin II. B. Hellman and F.V. Brchhansen, editors, Handb. Exp. Pharm. 32: 413-434.

Somogyi, M. (1945). Determination of blood sugar. J. Biol. Chem. 160: 69-73.

Steele, R. (1959). Influences of glucose loading and of injected insulin on hepatic glucose output. Ann. N.Y. Acad. Sci. 82: 420-430.

Steiner, D.F. and Oyer, P.E. (1967). The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc. Natl. Acad. Sci. U.S.A. 57: 473-480.

Stevenson, R.W., Steiner, K., Davis, M. Hendrick, G., Williams, P., Lacy, W. and Cherrington, A.D. (1983). Similar dose responsiveness of hepatic glycogenolysis and gluconeogenesis to glucagon. Fed. Proc. 42: 673.

Stryer, L. (1975a). Excitable membranes. In. Biochemistry, San Fransisco, W.H. Freeman and Co., pp. 787-806.

Stryer, L. (1975b). Glycogen and disaccharide metabolism. In. Biochemistry, San Fransisco, W.H. Freeman and Co., pp. 378-403.

Stryer, L. (1975c). Glycolysis. In. Biochemistry, San Fransisco, W.H. Freeman and Co., pp. 277-306.

Stryer, L. (1975d). Pentose phosphate pathway and gluconeogenesis. In. Biochemistry, San Fransisco, W.H. Freeman and Co., pp. 356-377.

Taunton, O.D., Stifel, F.B., Greene, H.L. and Herman, R.H. (1972). Rapid reciprocal changes of rat hepatic glycolytic enzymes and fructose-1, 6-diphosphatase following glucagon and insulin injection in vivo. Biochem. Biophys. Res. Commun. 48: 1663-1670.

Unger, R.H. (1983). Insulin-glucagon relationships in the defense against hypoglycemia. Diabetes 32: 575-583.

Unger, R.H., Madison, L.L. and Muller, W.A. (1972). Abnormal alpha cell function in diabetes. Diabetes 21: 301-307.

Unger, R.H. and Orci, L. (1981). Glucagon and the A Cell. New Engl. J. Med. 304: 1518-1524 and 1575-1580.

Valverde, I., Vandermeers, A., Anjaneyulu, R. and Malaisse, J. (1979). Calmodulin activation of adenylate cyclase in pancreatic islets. Science 206: 225-227.

Vranic, M., Gauthier, C., Bilinski, D., Wasserman, D., El-Tayeb, J., Hetenyi, Jr., G. and Lickley, H.L.A. (In press). Catecholamine responses and their interactions with other glucoregulatory hormones. Am. J. Physiol.

Vranic, M., Pek, S. and Kawamori, R. (1974). Increased "Glucagon Immunoreactivity" in plasma of totally depancreatized dogs. Diabetes 23: 905-912.

Walkenbach, R.J., Hazen, R. and Larner, J. (1978). Reversible inhibition of cyclic AMP-dependent protein kinase by insulin. Mol. Cell. Biochem. 19: 31-

Wollheim, C.B., Blondel, B., Trueheart, P.A., Renold, A.E. and Sharp, G.W.G. (1975). Calcium-induced insulin release in monolayer culture of the endocrine pancreas. Studies with ionophore A 23187. J. Biol. Chem. 250: 1354-1360.

Woods, S.C. and Porte, Jr., D. (1974). Neural control of the endocrine pancreas. Physiol. Rev. 54: 596-619.

Wool, I.G. (1975). Effects of insulin on cellular protein synthesis. In. Insulin II, B. Hellman and F.V. Bruchhausen, editors. Handb. Exp. Pharm. 32: 268-302.

APPENDIX A

Glucoregulatory Responses to Hypoglycemia and Hyperinslinemia in Normal Dogs

The following tables are a summary of figures published by Gauthier et al., 1980, Gauthier and Hetenyi, 1982 and Gauthier et al., 1983. The numbers under the headings "Phlorizin" and "Insulin" refer to the maximum or minimum values of the parameters during the respective infusions.

Table 6

Plasma concentration of glucose, glucagon and the rate of glucose production of post-absorptive dogs during the infusion of 50 $\mu g.kg^{-1}.min^{-1}$ phlorizin

	Experiment	Basal	Phlorizin	% Change
Glucose ($mg.dl^{-1}$)	A	107±9	~92	-16%
	B	112±2	~96	-16%
	C	105.6±0.7	~92	-12%
Glucagon ($pg.ml^{-1}$)	A	~70	~130	+85%
	B	72±4	~115	+59%
	C	84±9	~140	+67%
Ra ($mg.min^{-1}.kg^{-1}$)	A	2.9±0.5	~5	+72%
	B	3.2±0.1	~5	+56%
	C	2.7±0.7	~5	+85%

Experiment A : from Gauthier et al., 1980

B : from Gauthier and Hetenyi, 1982

C : from Gauthier et al., 1983

~ : Appoximately

Table 7

Plasma concentration of glucose, glucagon and the rate of glucose production of post-absorptive dogs during the infusion of 7 $mU.kg^{-1}.min^{-1}$ insulin

	Experiment	Basal	Insulin	% Change
Glucose	D	110±8	~50	-55%
($mg.dl^{-1}$)	E	108±7	~50	-54%
Glucagon	D	~65	~175	+169%
($pg.ml^{-1}$)	E	~100	~275	+175%
Ra	D	3.1±0.4	~6	+92%
($mg.min^{-1}.kg^{-1}$)	E	3.0±0.6	~5.5	+84%

Experiments D, E : from Gauthier et al., 1980

~ : Approximately

APPENDIX B

Multiple Comparisons in the Analysis of Variance

The null hypothesis H_0 of the analysis of variance is: The means of all the samples being compared are equal. The alternative hypothesis H_1 is: At least one sample has a mean which is significantly different from the rest. If the F ratio of the analysis of variance is greater than the set critical value, the null hypothesis is rejected and the alternative hypothesis is taken to be true. The analysis does not indicate which sample or samples are different. Statistical tests for multiple pairwise comparison must be carried out to determine which samples are different. These tests use the overall variance of all the data used in the analysis of variance. The use of this variance ensures that the level of significance of all the pair wise comparisons is the same as that of the overal null hypothesis of the analysis of variance. Tukey's Honestly Significant Difference, the Scheffe test and the Dunnett test were used in this study (Rosco, 1975, Dunnett, 1955 and Keppel, 1973).

1) Tukey's Honestly Significant Difference: This test is used for making all possible pairwise comparisons among means of samples of equal size. It uses a studentized range

stastisic. The test statistic is:

$$q = \frac{M_1 - M_2}{\sqrt{MS_E/n}}$$

where

M_1 and M_2: means of the samples compared

n: sample size

MS_E: Mean square of the error component of the analysis of variance (the denominator of the F ratio)

The critical value of q depends on k the total number of samples compared in the analysis of variance and the degree of freedom (d.f.) of MS_E.

2) The Scheffe Test is used for making all possible pairwise comparisons among means of samples of unequal size. The test statistic is:

$$F = \frac{(M_1 - M_2)^2}{MS_E\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(k - 1)}$$

with d.f. k - 1, a

where

M_1 and M_2: means of the samples compared

n_1 and n_2: sample size of samples compared

MS_E: mean square of the error component of the analysis of variance.

k: total number of samples in the analysis of variance

a: d.f. of MS_E

3) The Dunnett Test is used to compare one control sample to all the others. The test statistic "t" has a Dunnett t distribution (Dunnett, 1955) which is dependent on the total number k of samples compared in the analysis of variance. Dunnett's t is calculated as follows:

$$t = \frac{M_1 - M_2}{\sqrt{MS_E \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

with d.f. = a

M_1: mean of control sample

M_2: mean of the sample compared to control

n_1: size of control sample

n_2: size of compared sample

MS_E: mean square of the error component of the analysis of variance

a: d.f. of MS_E