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ABSTRACT

In non-anesthetized dogs the infusion of phlorizin decreases the level of plasma glucose from a mean 108 ± 9 to 86 ± 3 mg/dl. A more extensive decrease is prevented by a significant increase in the rate of glucose production, which is mediated by an increased concentration of glucagon in the plasma. When the increase of plasma glucagon is prevented by the simultaneous infusion of 0.35 μg/kg. min. somatostatin the increase in the rate of glucose production is also abolished and the concentration of glucose falls to 59 mg/dl. During the infusion of 7 mU/kg. min insulin the plasma glucose concentration is reduced to 49 ± 3 mg/dl. Both the plasma glucagon concentration and the rate of glucose production increased. Somatostatin abolished the rise in the concentration of glucagon in the plasma, but has no effect on the markedly increased rate of glucose production. When somatostatin was infused both during and after the infusion of insulin, the rise in the concentration of glucose after the cessation of the insulin infusion was somewhat slower than in dogs to which no somatostatin was infused. Conclusion: Relatively small deviations from the set point of plasma glucose concentration release glucagon which, in turn, increases the rate of hepatic glucose

production. Judged by the change in plasma free fatty acids concentration, epinephrine does not seem to be involved in this response. In overt hypoglycemia complete suppression of glucagon release does not even diminish the increase in hepatic glucose production. It is suggested that in the resting state glucagon is a physiological regulator of plasma glucose in the hypoglycemic range, whereas the release of epinephrine represents an emergency mechanism activated by cerebral glucopenia.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. G. Hetenyi under whose supervision this work was conducted. His patient guidance, understanding and foresight were invaluable.

Many thanks to Dr. M. Vranic, University of Toronto, for his valued criticisms, his judicious advice concerning the third series and the technical assistance of his laboratory for IRG and GLI determination.

I am grateful to Dr. S. Raman for the statistical analysis of the experiments of the third series.

I am also much obliged to Dr. R. Deghenghi, Director of Research, Ayerst Laboratories (Montreal) for his generous gift of somatostatin.

My thanks to Mrs. I. Popescu. B.Sc., Mrs. C. Ferrarotto and Mrs. N. Kovacevic, P. Eng. for their excellent technical assistance. The assistance of Mr. J. Anthony was also appreciated.

The aid of Mr. R. Layberry in the areas of programming was also greatly appreciated.

It is with pleasure that I acknowledge the financial support of the Medical Research Council of Canada (MT1038). Without their support this work could not have been carried out.

TABLE OF CONTENTS

LIST OF FIGURES

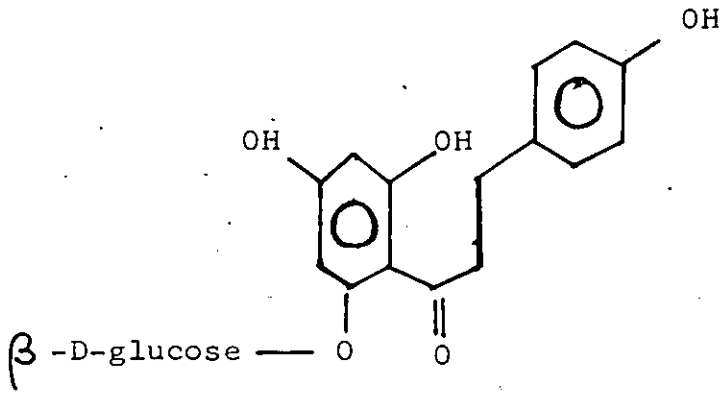
LIST OF TABLES

CHAPTER I

INTRODUCTION

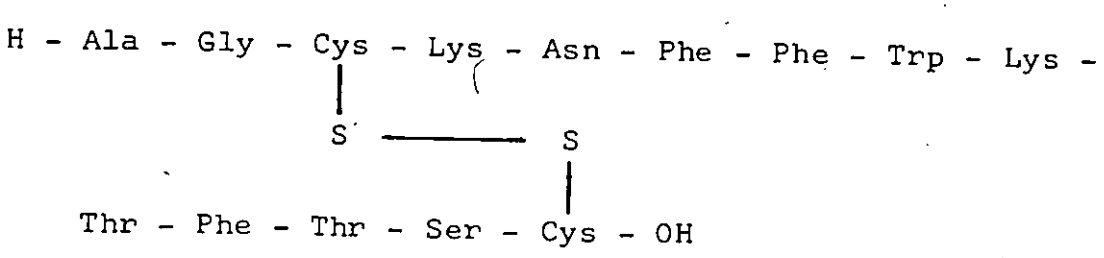
It is a well established fact that in insulin-induced hypoglycemia the rate of hepatic glucose production increases (Hetenyi et al, 1961; De Bodo et al, 1963). This hepatic response to hypoglycemia is a regulatory homeostatic mechanism, an attempt to restore the normal level of glucose in the plasma. Because of the large amount of insulin infused to keep the concentration of glucose in the plasma low, and due to the well-known "restraining" effect of insulin of hepatic glucose output (Dunn et al, 1959), the response does not achieve its goal. The increased output of glucose represents a maximal response by the liver in a system that has failed to maintain its set point: the normal level of glucose in the blood, because of an excessive outside disturbance.

Phlorizin is the glycoside of a polyhydroxyphenol present in the barks of several fruit trees, and has the following structural formula:



It is the most potent non-competitive inhibitor of glucose reabsorption in the kidney tubules and its receptor has been shown to be either identical to or part of the glucose transporter exposed at the exterior face of the brush border membrane of the proximal tubule in dog kidney (Silverman and Black, 1975). As early as 1927, phlorizin was shown to produce prompt and persistent glucosuria without hypoglycemia (Nash, 1927). This glycoside appeared to be a useful tool for studying physiological processes involved in regulation of blood glucose due to the fact that the increased hepatic glucose output is due only to an increased removal of glucose from blood by glucosuria (Kolodny et al, 1962) and that, in proper doses, phlorizin does not interfere with hepatic glucose production (Goresky et al, 1974).

In 1972, a somatotropin-release-inhibiting factor was isolated from the ovine hypothalamus by Brazeau et al and named "somatostatin". The structural formula of somatostatin, as established by Brazeau et al (1973), is the following:



There is also a linear form of this factor with the same biological activity than the cyclized natural form of the peptide. The tetradecapeptide has an estimated biological half-life of 2-4 minutes in circulation in the rat (Vale et al, 1975). It was also shown to inhibit insulin secretion in man (Alberti et al, 1973) and glucagon secretion in the rat pancreas perfused in situ (Johnson et al, 1974). More recently, this peptide was shown to suppress glucagon-stimulated glucose release from rat liver in vitro in a dose-dependent manner (Sacks et al, 1977). However, it was shown that the acute changes in the plasma level and turnover of glucose induced by somatostatin are attributable to its effect on the endocrine system and not to any direct effect on glucose metabolism (Cherrington et al, 1977).

The purpose of the experiments to be reported is to test the glucoregulatory system: (a) in the absence of an excessively high plasma level of insulin; (b) when it is successful in compensating for a hypoglycemic stimulus. In other words, when it maintains a normal level of glucose in the plasma in face of a moderately increased rate of removal of glucose from the circulation.

In particular, answers to four questions were sought. (a) Does increase in hepatic glucose production match the increase in the rate of glucose disappearance from the plasma and, if so, with what precision ? (b) To what extent is glucagon responsible for the increase in hepatic glucose production during the infusion of phlorizin and during insulin induced hypoglycemia ? (c) Are there any emergency mechanisms activated in overt, insulin induced hypoglycemia, i.e. mechanisms which are not operative in the physiological regulation of plasma glucose around the set point ? (d) And finally, what is the relative importance of an increased secretion of glucagon in the rate of recovery of normoglycemia when an infusion of insulin has been discontinued ?

CHAPTER 11

REVIEW OF THE LITERATURE

A. HYPOGLYCEMIA

History

Interest in hypoglycemia arose with the observation, by Mann and Magath in 1922, that cerebral dysfunction resulted from a fall in the blood sugar level produced by hepatectomy. In the adult, the commonest cause of hypoglycemia is an overdose of insulin, recognized as early as 1922, when insulin therapy for diabetes mellitus was introduced.

Definition

In simple terms hypoglycemia means a situation where the concentration of glucose in blood is below that considered to be "normal". Hypoglycemia is the result of excessive glucose removal from the circulation over release of glucose into it. However, in hypoglycemia when plasma glucose concentration is at a steady but low level, the ratio of glucose release and removal are equal. Restoration of normal plasma glucose level is achieved by a period during which the rate of release of glucose into the circulation exceeds the rate of removal.

Classification

Three ways of classifying hypoglycemia are

generally recognized: aetiological, clinical and physiological. In the context of the present studies, being based on the turnover of the glucose pool, the physiological classification seems the most appropriate.

According to this classification hypoglycemia can be the result of (1) increased outflow from the glucose pool which is inadequately or not at all compensated for by an increase in inflow. Hypoglycemia due to excessive exercise and overdose of insulin falls into this category. (2) The inflow of glucose, i.e. the rate of hepatic glucose production, is reduced. This is the case in some types of liver disease, hypopituitarism or starvation. (3) Marks and Rose (1965) list hypoglycemia due to insulinoma or non-pancreatic tumors into a separate category. Actually hypoglycemia caused by an excessive amount of insulin falls into the first category, whereas hypoglycemia due to non-pancreatic tumors are believed to be due to a combination of (1) and (2).

B. REGULATORS OF GLUCOSE HOMEOSTASIS

The liver

The central role of the liver in maintaining carbohydrate homeostasis is well recognized. Its primary function in this regard is to provide adequate supplies of glucose to

tissues that utilize it as a metabolic fuel. In the brain, red blood cells and the renal medulla glucose is the main if not the only fuel. The main features of carbohydrate metabolism in the liver are thus the formation, storage and release of glucose, so that the liver acts as a blood "glucostat". Figure 1 presents the role of the liver in carbohydrate metabolism.

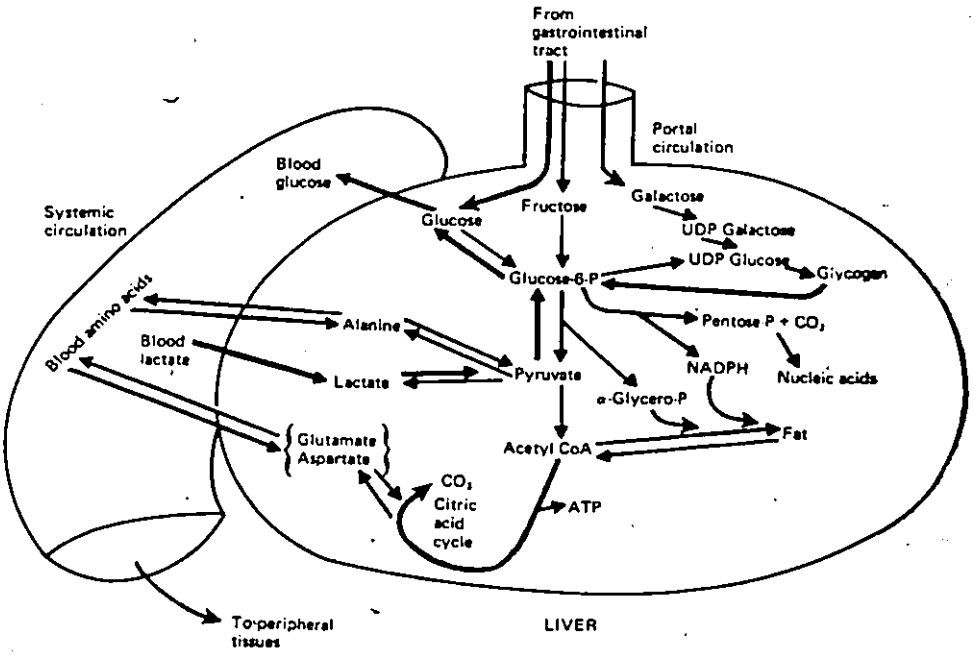


Figure 1: Role of the liver in carbohydrate metabolism (McMurray, 1977).

From the intestinal-lining cells where they are absorbed, the ingested sugars diffuse into blood capillaries draining into the portal vein to be finally taken up by the liver cells. This is done rapidly due to the fact that, unlike most cells, hepatocytes are freely permeable to glucose (Cahill et al, 1958). It must also be added that the liver is a very versatile organ, so that it does not only retain and store glucose from dietary sources but is also able to manufacture it from other carbohydrates such as fructose, galactose, pyruvate and lactate as well as from noncarbohydrate sources such as glycerol, dicarboxylic acids and certain amino acids.

In the absence of barriers to permeability, the ultimate fate of glucose in the liver is determined by enzymes. As in other tissues the first step in the metabolism of glucose is phosphorylation to its non-diffusible form, glucose 6-phosphate. The enzyme involved is a hexokinase having a high affinity for glucose and operating near maximal rates at 0. 002-0.005 M glucose concentrations (36-90 mg/dl) (Altszuler and Finegold, 1974). Due to the lack of accumulation of radioactive glucose during 6 hr infusions of trace amounts of ^{14}C-glucose in the liver of dogs fasted for 18 hours (Bishop et al, 1965), it is evident that there is no uptake in circulating plasma glucose by

the liver in post-absorptive state, i.e. 18-24 hours following a meal. At this step, the pancreatic hormone insulin can contribute to the retention of glucose by the hepatic cells by causing an increased synthesis of the phosphorylating enzyme glucokinase. In the liver, glucose 6-phosphate may either be incorporated into glycogen or metabolized via three different ways.

Glucose 6-phosphate may give rise to pyruvate by glycolysis. Pyruvate then enters the tricarboxylic acid cycle. This constitutes the first pathway and provides the major source of energy for liver metabolism. The second pathway provides an alternate but essential route for the degradation of glucose 6-phosphate; it is the pentose phosphate pathway, the various reactions in which can be divided into oxidative and non-oxidative types. The oxidative steps lead to the formation of $NADPH_2$ which is essential for the synthesis of fatty acids, bile salts and steroids and for the reduction of oxidized glutathione. The non-oxidative reactions are believed to form pentose phosphate which is utilized in the formation of ribonucleic acid . The third pathway is the glucuronic acid pathway and results in the formation of D-glucuronic acid. This pathway is important in that UDP-glucuronic acid has a high free energy of hydrolysis providing energy for the

reactions in which the glucuronic acid participates such as: incorporation into mucopolysaccharides and conjugation of steroids, bile salts, and numerous drugs in preparation to their excretion.

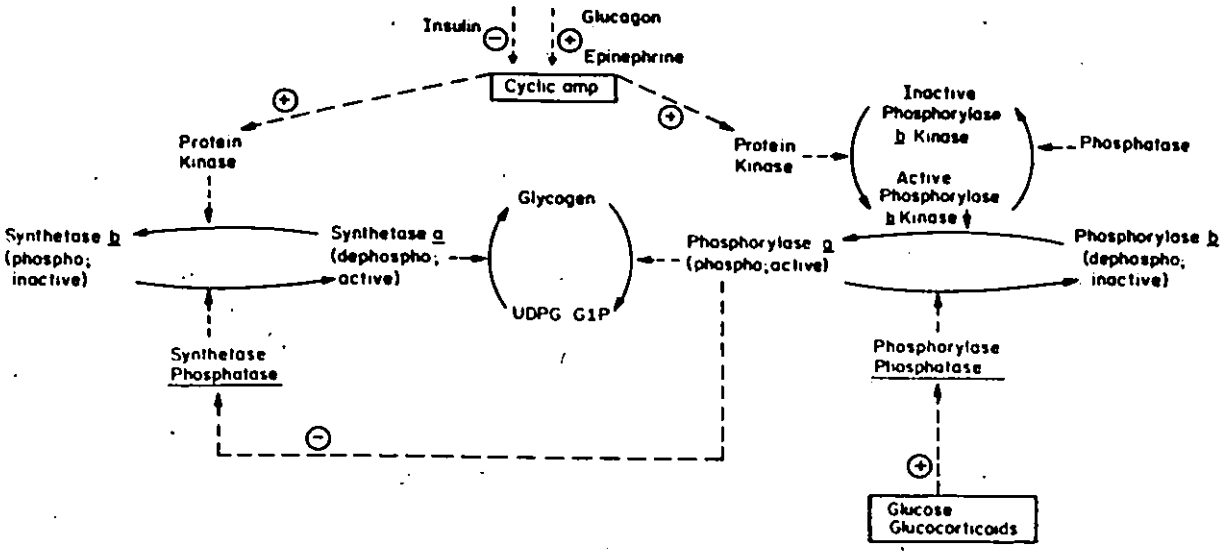


Figure 2: Regulation of glycogen metabolism in the liver (Altszuler and Finegold, 1974).

Glucose 6-phosphate is converted and stored as the insoluble polymer glycogen. Liver glycogen constitutes a rapidly mobilizable source of sugar which can meet the carbohydrate needs during the first 18-24 hours in man

(McMurray, 1977) and in dogs (Bishop et al, 1965) after the withdrawal of food. Figure 2 presents schematically the regulation of glycogen metabolism. As shown on this figure, the rate-limiting enzyme for glycogen synthesis is synthetase a and the one for glycogenolysis is phosphorylase a. The activation of these two enzymes depends in turn on a further interplay of various influences such as enzymes, salts, substrates and hormones. Regulatory influences can therefore be initiated at a number of sites that directly or indirectly affect the rate-limiting enzymic reactions.

The regulators

Hepatic glycogen synthesis is increased by glucose, insulin, glucocorticoids, and growth hormone. Glucose alone may increase glycogen synthesis, both in vitro and in vivo by activating synthetase, whereas other sugars do not (Hers et al, 1970). As shown on figure 2, the activation of synthetase by glucose is probably brought about indirectly through its effect on phosphorylase phosphatase (Hers et al, 1970). Insulin also increases glycogen synthesis. The infusion of insulin, along with enough glucose to prevent hypoglycemia in the normal dog, increases liver glycogen formation within the first hour of infusion (Bishop et al, 1965). This effect of insulin

may be caused in part by a lowering of hepatic cyclic adenosine monophosphate (c-AMP) (Jefferson et al, 1968), thereby deactivating protein kinase, as shown on figure 2. Another possible explanation would be the inhibition of the responsiveness of protein kinase to c-AMP by insulin (Shen et al, 1970). Glucocorticoids activate liver synthetase and increase glycogen indirectly by increasing synthesis of phosphorylase phosphatase. The presence of glucocorticoids is also required for activation of phosphorylase by glucagon, epinephrine and c-AMP (Exton et al, 1970), suggesting a "permissive" action of glucocorticoids at some site prior to c-AMP. Administration of growth hormone leads to high concentrations of glycogen in the liver of rats and dogs (de Bodo and Altszuler, 1957; Bishop et al, 1967).

As shown on figure 2, glycogenolysis is also affected by glucagon and epinephrine. Both agents increase the synthesis of c-AMP through their action on adenylcyclase. The intracellular c-AMP, in turn, stimulates a protein kinase, which exerts two complementary effects on glycogen: it activates phosphorylase kinase, thus increasing glycogenolysis, and it inactivates synthetase, thereby inhibiting glycogen synthesis. Hypoglycemia stimulates glycogenolysis and increases hepatic glucose output. It is generally assumed that glucagon and epinephrine are responsible for

this effect. However, following the lowering of plasma glucose, four different hormones, acting either alone or in concert, are capable of changing the metabolism of hepatic, adipose and muscle cells so as to prevent further decrease of plasma glucose concentration or to restore it to its normal level. These hormones are: growth hormone, cortisol, epinephrine and glucagon.

C. ENDOCRINE REGULATORS OF HYPOGLYCEMIA

Growth hormone

Growth hormone (GH) is produced by the anterior pituitary. Its secretion is under hypothalamic control, a growth hormone releasing substance being secreted by the hypothalamus into the hypothalamico-hypophysial portal system by which it reaches the anterior pituitary where it induces the production of GH (Reichlin, 1963). The GH releasing factor has not been isolated as of to-day. An alternative regulation of GH secretion may be by the secretion of a GH releasing inhibitory substance also called somatostatin. This substance was also isolated from the ovine hypothalamus (Brazeau et al, 1973) following consistent observation that some ovine hypothalamic extracts inhibited the *in vitro* secretion of GH. Somatostatin-like activity (SLA) has been shown to be anatomically located

throughout the brain, spinal chord, gastrointestinal tract and pancreas (Vale et al, 1977). However, no physiological role has yet been proven for somatostatin.

In 1958, De Bodo and Altszuler demonstrated conclusively that GH is a physiological insulin antagonist. They found that very small doses of bovine GH to hypophysectomized dogs ameliorate their insulin hypersensitivity. Even though GH secretion is inversely proportional to the concentration of plasma glucose (Marks and Rose, 1965), it has been demonstrated that it is not simply the alterations in the blood glucose which changes the secretion of GH. Indeed in man the utilization of glucose by moderate exercise, unaccompanied by a detectable change in blood sugar concentration, was accompanied by a significant increase in plasma GH secretion (Roth et al, 1963). Beginning at around 1966, different groups of researchers investigated the acute effects of relatively large doses of GH. Following a single 3 mg/kg injection of GH in dogs, Campbell and Rastogi (1966) observed a fall in serum free fatty acids (FFA) during the first 30 to 120 minutes. They also found that this fall was not associated with change in serum glucose or insulin concentrations, showing that this initial effect of GH on FFA is not dependent on insulin. With daily administration of GH for 4 days, they observed an elevation

in serum insulin and an augmented increase in serum insulin in response to glucose correlated with high concentrations of FFA. In man, the first and most consistent effect of GH is also an increase in FFA in the blood, this response beginning 2 hours after the injection of GH (Raben and Hollenberg, 1959). Hypoglycemia is a particularly potent stimulus to increase GH secretion. In terms of moment-to-moment regulation of plasma glucose, it is rather counter-productive to increase the secretion of an hormone which eventually causes an increased secretion of insulin in response to a low concentration of glucose in the plasma. However, in terms of a long term, integrated response to fasting, GH seems to switch over slowly the source of fuel for the body from carbohydrate to fat. It was only following the discovery of an hypothalamic polypeptide inhibiting the secretion of GH, somatostatin (Brazeau et al, 1973), that the situation regarding GH was clarified.

Taking advantage of this property of somatostatin, Gerich et al (1976) suppressed the endogenous secretion of GH in juvenile-onset, insulin dependent diabetics and restored physiologic circulating levels of this hormone by an exogenous infusion. He was able to show that under appropriate conditions, physiologic levels of GH can augment lipolysis and ketonemia in man, but these actions

are ordinarily not apparent in the presence of normal levels of insulin.

Formerly known under the operational term "sulfation factor" or "thymdine factor", the somatomedins are a group of polypeptides released by the liver (McConaghey and Sledge, 1970) which have been demonstrated to be under the control of GH (Salmon and Daughaday, 1956; Daughaday and Reeder, 1966). These substances also possess insulin-like activity in the presence of insulin antibodies and they are mitogens. Even though somatomedins are believed to mediate the biologic actions of GH on some of its target tissues (Salmon and DuVall, 1970), direct proof of this "somatomedin hypothesis" is still lacking.

Cortisol

Cortisol, cortisone and corticosterone are steroid hormones secreted by the adrenal cortex that participate in the regulation of glucose metabolism. In the dog cortisol is the major glucocorticoid. It is released from the adrenal cortex in response to adrenocorticotropic hormone (ACTH). ACTH stimulates specific membrane receptors on the cells of the zonae fasciculata and reticularis. This results in an increased adenylate cyclase activity and an increased conversion of cholesterol to cortisol in these cells.

Cortisol controls its own secretion. There is a negative feedback between the concentration of cortisol in the plasma and the secretion of the hypothalamic neurosecretory hormone, corticotropin-releasing hormone (CRH), which stimulates the release of ACTH from the anterior pituitary. Cortisol secretion is also under direct neural control, stressful stimuli such as a hemorrhage or hypoglycemia stimulate the secretion of ACTH (Selkurt, 1975).

In the early studies of Long and Lukens (1935) and Britton and Silvette (1932) defects in carbohydrate metabolism due to adrenal deficiency in experimental animals were recognized. However, even with adrenalectomized animals showing altered metabolic responses to provocative stimuli such as an injection of insulin, the abnormalities are not evident. Rates of glucose production and utilization in the conscious adrenalectomized dog, measured by tracer methods,were found to be normal in the post-absorptive state (Altszuler, 1975). In the same way, in normal rats (Ingle and Baker, 1953) and normal dogs (De Bodo et al, 1953), doses of cortisone that are high enough to maintain adrenalectomized animals have been shown to have little effect . It has also been shown that the primary action of cortisol is to enhance protein breakdown in extrahepatic tissues (Thorn, 1959) in order to provide gluconeogenic

precursors and to stimulate the slower synthesis of gluconeogenic enzymes in the liver.

Evidence for an effect of cortisol on peripheral glucose utilization is conflicting. Indeed in 1965, based upon experiments of Lecocq , Marks and Rose (1965) stated that the earliest and most important action of cortisol was to inhibit glucose metabolism. However 3 daily injections of methylprednisolone (MP) increase overall glucose uptake (Rd) in normal dogs (Ninomiya et al, 1965) and enhance removal of an administrated glucose load (Campbell and Rastogi, 1968). It seems that because of the effect of MP on the release of insulin, the effect of glucocorticoids on overall glucose utilization in the normal dog would appear to be one of stimulation rather than of inhibition(Altszuler et al, 1973).

Taking into account the facts that undisturbed adrenalectomized animals in the post-absorptive state do not show evident abnormalities in terms of rates of glucose production and utilization and that cortisol stimulates the slow process of synthesis of hepatic gluconeogenic enzymes (Long et al, 1940), it seems obvious that cortisol is not a minute-to-minute regulator of blood glucose. Its role is more in the defence against hypoglycemia during starvation when hepatic glycogen stores have been

depleted and glucose production is entirely by gluconeogenesis.

There is accumulating evidence to the effect that neither cortisol or GH play a causative role in the counterregulation of hypoglycemia. Using 2-deoxy-D-glucose-induced glucopenia in normal and adrenal medulla denervated patients, Brodows et al (1973) showed that only the normal but not the operated subjects had a rise in plasma glucose concentration, even though both groups had a brisk rise in plasma cortisol and GH. Only the normal subjects had a rise in plasma epinephrine, suggesting this hormone as the major regulator of intracellular glucopenia, even though glucagon level was not measured in this experiment. Using oral doses of the serotonin antagonist cyproheptadine to block the hypoglycemia-induced increase in plasma levels of cortisol and GH by 81 and 73% respectively, Feldman et al (1975) showed that increased secretion of these hormones is not essential for the counterregulatory rise in plasma glucose following insulin-induced hypoglycemia. According to DeFronzo et al (1977) who studied counter-regulatory hormonal response in man following a rapid fall in blood glucose, a mean decrease in blood glucose of 28 mg/dl below basal levels occured before GH was released and 39 mg/dl before cortisol was released. It is thus

unlikely that either of these hormones plays an important role in the moment-to-moment control of physiologic changes in blood glucose concentration. They rather seem to be involved indirectly in the actions of faster acting endocrine regulators, such as the lipolytic and glycogenolytic effects of cathecolamines, which are dependent on the presence of adequate amounts of cortisol; these permissive actions have already been mentioned in the section on the liver. Some permissive action also takes place between the two hormones and insulin, since De Bodo and Altszuler (1958) showed that basal cortisol and GH secretion are essential for the maintenance of normal insulin sensitivity for adrenalectomized or hypophysectomized dogs have striking insulin hypersensitivity. Finally, interaction between cortisol and GH is quite unlikely, since it was shown, looking at spontaneous variation of cortisol and GH secretion in adolescents that physiological fluctuations of cortisol do not alter GH secretion (Muehlendahl et al, 1978).

Epinephrine

The first hormone whose structure was established, epinephrine (or adrenaline), in a sense, may be said to have been the starting point of modern endocrinology. The

early recognition of its structure, as well as the availability of histological, biological and chemical methods for its detection are responsible for the vast literature extant on this hormone (Axelrod, 1962). Even though there is no doubt now that epinephrine is a hormone, its hormonal activity was questioned in the past (Gley and Quinquad, 1918) due to its very low concentration in the blood in the resting state.

After complete adrenalectomy in experimental animals (Del Basso et al, 1970) or man (Elmadjian et al, 1956), the urinary excretion of epinephrine falls to very low levels. This proves that most of the epinephrine is synthesized by the adrenal medulla. The pathway for this synthesis has been elucidated by Blaschko (1939).

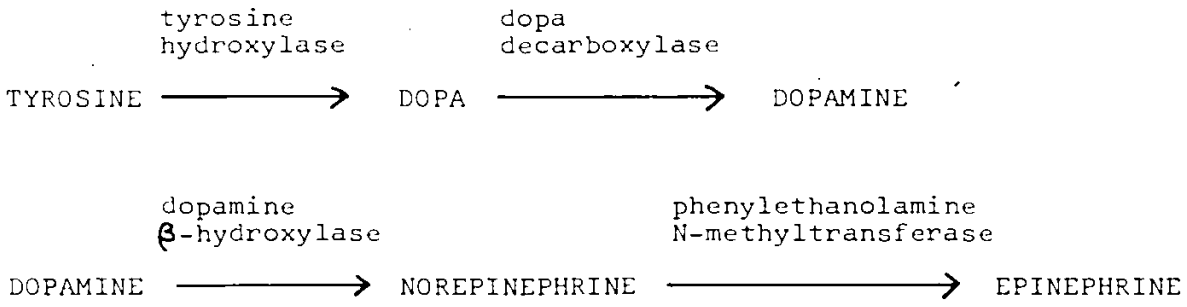


Figure 3: Biosynthesis of epinephrine (Fuller, 1973).

As shown on Figure 3 four steps are involved in the synthesis

of epinephrine: hydroxylation of tyrosine, decarboxylation of DOPA, β-hydroxylation of dopamine and methylation of norepinephrine. Like any other enzymic process, the synthesis of the adrenomedullary hormone may be limited or regulated by two factors: the availability of substrate and the activity or amount of the enzymes involved. Due to the fact that the concentration of tyrosine exceeds the affinity constant (K_m) of tyrosine hydroxylase, which is the rate-limiting enzyme (Nagatsu et al, 1964), precursor availability does not seem to be a control point in the regulation of cathecolamine synthesis in the adrenals. According to Fuller (1973), it is most likely that changes in enzymic activity are involved in short term regulation, whereas changes in the amounts of the enzymes are important in the long-term regulation of epinephrine synthesis.

In relation with short term regulation, three sites are available for endogenous inhibition: (a) the tyrosine hydroxylation, (b) the dopamine-β-hydroxylation and (c) the methylation of norepinephrine. The first step is inhibited by cathecolamines themselves (Nagatsu, 1964; Udenfriend, 1965). Indirect evidence for possible inhibition at the dopamine-β-hydroxylase step is given by the natural occurence of an inhibitor of that enzyme in the adrenal

medulla (Duch and Kirshner, 1971). This inhibitor is inactivated by sulfhydryl-binding reagents and appears to be a sulfhydryl-containing compound resembling glutathione in amino acid composition. According to Bülbring (1949) the rate of epinephrine synthesis from norepinephrine is higher in glands in which the store of epinephrine has been depleted by previous splanchnic stimulation. This may be interpreted as an inhibition of methylation by epinephrine itself. Nevertheless, since most of the epinephrine is stored in granules and is not able to interact with the enzyme phenylethanolamine N-methyltransferase (PNMT), this product inhibition is difficult to interpret.

The three enzymes involved in the synthesis of cathecolamines are under hormonal and neural control. In 1951, Shepherd and West suggested that "the adrenal cortex is possibly concerned with methylation of norepinephrine". A factor secreted by the cortex and associated with methylation of norepinephrine was suggested by Coupland (1953) two years later. This factor was identified as a glucocorticoid in 1965 by Wurtman and Axelrod who have also shown that suppression of adrenocortical output in the rat by hypophysectomy was acting by feedback inhibition of ACTH and causing a reduction of PNMT in the adrenal glands. That the increase in PNMT produced by glucocorticoids was

not dependent of neural inputs was shown by the fact that denervation of the adrenals in hypophysectomized rats did not prevent the increase of PNMT by ACTH (Kvetnansky et al, 1970). Altogether, these studies demonstrated, for the first time, the direct control of a key enzyme, PNMT, in the epinephrine synthesis by cortical hormones (Fuller, 1973). In contrast, the other two enzymes, even though their amounts in the adrenal cortex are reduced following hypophysectomy (Kvetnansky et al, 1970;Gerwirtz et al, 1971), appear to be regulated mainly by neural mechanism (Gerwirtz et al, 1971; Silbergeld et al, 1971). Indeed, although both drugs and hormones may influence the secretion of epinephrine, the physiological control of its secretion is neural and wherever the impulses of that signal for epinephrine release originate, they reach the adrenal via neurones from the lower thoracic and the lumbar sympathetic region of the spinal chord (Fuller, 1973).

Among the stimuli to epinephrine secretion, hypoglycemia has been studied most often. A neural mechanism for epinephrine secretion following insulin-induced hypoglycemia was first proposed in 1924 by Cannon et al, following the observation that adrenal denervation blocks the response (Fuller, 1973). These early experiments used the denervated heart of cats in situ for the assay of epinephrine. More recent evidence

was provided in man (Vendsalu, 1960) and in dogs (Goldfien et al, 1958) from direct measurements of plasma cathecolamines following insulin-induced hypoglycemia. It may also be noted that epinephrine levels do not rise in response to a mild decline of blood glucose, Goldfien et al (1958) having observed no significant rise in epinephrine concentration before reaching 45-50 mg/dl in the lightly anesthesized dog. Similarly, earlier observations by De Bodo and Sinkoff in 1953 (Altszuler, 1975; Cryer, 1976) that the adrenal denervated dog could still maintain a normal blood glucose level when subjected to mild exercise were in agreement with Goldfien's results.

The infusion of epinephrine at a rate of 0.1 μg/kg.min caused in dogs a prompt rise of 12-15 mg/dl in plasma glucose by 15 minutes and a further slow rise by 30-35 mg/dl above control values at 60 minutes. This was followed by period of a constant plasma glucose level (Altszuler et al, 1967). The concentration of epinephrine in the plasma reached a level comparable to the one observed in insulin-induced hypoglycemia (Goldfien et al, 1958). As shown by Altszuler et al (1967 and 1975), epinephrine inhibits the release of insulin in response to glucose. Mechanisms proposed to explain this inhibitory effect include the inhibition of hexokinase following increase in intracellular

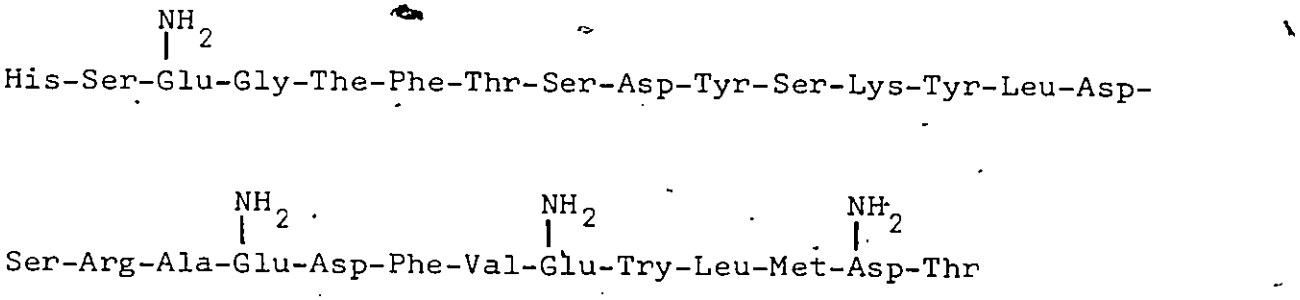
glucose 6-phosphate subsequent to glycogenolysis and the concept developed by Randle of the interference of FFA, released in response to lipolytic activity of epinephrine, with glucose uptake by inhibition of hexokinase and phosphofructokinase in various cells (Altszuler, 1975). For other species, it has been shown, using adrenodemedullated and reserpin treated rats, that the integrity of the sympathetic nervous system is essential for an efficient homeostatic response to insulin-induced hypoglycemia, and that glucagon hypersecretion by itself does not seem able to counteract the restraining effect on glucose production induced by pharmacologic doses of insulin (Sacca et al, 1979). However, the effects of epinephrine may be mediated through changes induced in pancreatic endocrine function, since it was shown that epinephrine stimulates glucagon release (Gerich et al, 1974).

Using glucagon blockade by somatostatin and epinephrine infusion at the same rates than those used previously in dogs (Altszuler, 1967) but in nonanesthetized baboons in post-absorptive state, Chideckel et al (1977) showed that the early epinephrine-induced glycogenolysis was mediated by epinephrine-induced glucagon release, but not the delayed hyperglycemia (Altszuler et al, 1967) and probably not the

lipolysis.

Glucagon

Murlin et al in 1923, first noted a transient hyperglycemic response to certain pancreatic extracts and suggested the name glucagon for the causative agent. Thirty years later, Staub et al (1953) isolated glucagon from an amorphous fraction of commercial insulin. Enzymic digestions, chromatographic analysis and electrophoresis revealed glucagon to be a straight chain polypeptide of 29 amino acids arranged in the following sequence:



Glucagon and insulin are related in many ways. Structurally, it will be noted that glucagon contains all the amino acids present in the insulin molecule, except proline, isoleucine and cystine and that it contains two amino acids not present in insulin, methionine and tryptophane. The anatomical location of most of the cells of their origin

is the same, the islets of Langerhans, even though there are extrapancreatic α-cells in the gastrointestinal tract that secrete glucagon. In the islets of most animal species, a rim of glucagon-secreting α-cells tends to surround nest of insulin-secreting β-cells. Somatostating-secreting D-cells are found in juxtaposition to the α-cells (Unger and Orci, 1976). By their glucoregulatory role, the opposing and counterbalancing actions of the two hormones, insulin and glucagon, allow for a fast and precise control over glucose flux. Based on the fact that the response of both α and β-cells to secretogogues or suppressant, other than glucose, can be elicited only when such a response would not result in undesirable hypoglycemia or hyperglycemia, Unger and Orci (1976) proposed that glucoregulation appears to take precedence over all other functions of α and β-cells. Glucose is the single most potent physiological glucagon suppressor as well as the most potent stimulus of insulin secretion. Conversely the lack of glucose is a potent stimulus for glucagon secretion and suppressor of insulin release. This bihormonal "push-pull" system thus becomes an important factor in the maintenance of the narrow range of glycemia that prevails in normal subject. Indeed, this idea that glucagon is as important as insulin in blood glucose homeostasis was directly demonstrated by Alford

et al (1974) who suppressed by an infusion of somatostatin the endogenous glucagon secretion and consequently plasma glucagon concentration to undetectable levels observed a fall in plasma glucose at the rate of 0.5 mg/dl.min. This was prevented by a glucagon infusion which restored the normal concentration of glucagon in the portal vein. However, in the same year, the transient nature of glucagon action was noted by Liljenquist et al (1974). They found that during the infusion of glucagon splanchnic cyclic-AMP production falls after an early peak, even in diabetics where there was no endogenous insulin secretion. Thus the effect of glucagon on carbohydrate homeostasis is on acute reactions. Its long term regulatory role however is doubtful.

Many factors stimulate secretion of glucagon: amino acids, cyclic-AMP, hypoglycemia, the gut hormones, gastrin, pancreozymine, cholecystikinin, gastric inhibitory polypeptides and neurotensin. On the other hand, glucose, fatty acids, ketone bodies and secretin inhibit secretion.

The liver is the major target organ of glucagon. It has been shown to be rich in glucagon receptors which are linked to adenylcyclase (Rodbell et al, 1971). Sutherland and Cori (1951) showed that glucagon stimulates hepatic glycogen breakdown. Miller (1960) showed that glucagon

stimulates gluconeogenesis in the isolated, perfused rat liver. Both effects are mediated by c-AMP. Other important hepatic actions include hepatic extraction of gluconeogenic precursors such as alanine, stimulation of ureagenesis, inhibition of hepatic protein synthesis, stimulation of ketogenesis and inhibition of lipoprotein secretion. In adipose tissue, glucagon stimulates lipolysis. This too is mediated by c-AMP. Finally, in contrast to epinephrine which stimulates glycogenolysis in muscle as well as in the liver, glucagon has no such effect on muscle (Alberti and Nattrass, 1977). The predominant effect of glucagon on the liver was clearly demonstrated by Vranic et al (1977). In depancreatized dogs infused with insulin and glucagon, the glucagon-induced increment in the rate of glucose production was not affected by insulin. Insulin decreased the concentrations of glucose in the plasma only by its peripheral effect in proportion to the rate of infusion.

To conclude, due to the predominant hepatic effect of glucagon over insulin and the transient nature of its action, it appears that: (a) the physiologic role of glucagon is to increase hepatic glucose production rapidly, and that (b) its transience could protect the liver against depletion of its glycogen stores.

D. THE USE OF SOMATOSTATIN IN GLUCOREGULATORY STUDIES

Somatostatin (SST) was found to lower basal level of insulin in the plasma and to suppress glucose-induced insulin release in man (Alberti et al, 1973). It has been shown to inhibit both basal and arginine-stimulated secretion of both insulin and glucagon in fasted baboons, dogs or rats (Koerker et al, 1974; Ruch et al, 1973). Johnson et al (1974) demonstrated conclusively that the inhibition of hormonal secretion is at the level of the islets cells. They found in perfused rat pancreas in situ, that there is a more than 50% fall in basal glucagon and insulin secretion after 10 minutes of perfusion with 100 ng/ml SST and that arginine-stimulated hormonal secretion of glucagon and insulin are completely suppressed. These observations were in agreement with those of Alberti et al (1973), who found SST to inhibit insulin secretion in the isolated perfused dog pancreas at a concentration as low as 1 ng/ml. Applying different acute and chronic stimuli of either glucagon or insulin secretion to fasted baboons with and without an infusion of linear SST, Chideckel et al (1975) studied the nature of SST-induced inhibition of pancreatic endocrine secretion. They concluded: that (a) SST is a potent and universally effective inhibitor of both the acute and chronic phases of stimulated insulin

and glucagon secretion, (b) these inhibitory effects are reversible and (c) the effect of SST on blood glucose seems to be mediated through its effect on blood glucagon, since the effect of the lowered plasma glucagon concentration on hepatic glucose production was not effectively counteracted by the simultaneous fall in plasma insulin. The main conclusion of most experiments in vivo seems to be that the action of SST is due to inhibition of pancreatic glucagon secretion. However, following the observation that addition of SST to isolated rat hepatocytes inhibited both glucagon-stimulated glycogenolysis and glucoenogenesis by 40-50%, Oliver and Wagle (1975) postulated that SST does not only inhibit the secretion of hormones from the islets of Langerhans but also interferes with the effect of glucagon on hepatic gluconeogenesis and glycogenolysis. Another study by Sacks et al (1977), using isolated perfused rat liver, seems to support this hypothesis. On the other hand, conflicting results were obtained by Chideckel et al (1975) and Oliver and Wagle (1975). In view of the important conclusions being drawn from studies using SST to assess the hormonal regulation of glucose metabolism, it was essential to determine whether or not the peptide exerts direct effects on the net flux of glucose into fat and muscle or out of the liver. Such a complete study was

accomplished by Cherrington et al (1977) using perfused rat hindquarters, isolated rat adipocytes and isolated rat hepatocytes. Their findings were in agreement with those reported by Chideckel et al (1975) in liver slices, showing that SST was without effect on basal or glucagon-stimulated glucose output. They also noted that studies of Oliver and Wagle are paradoxical since they observed an increasing inhibition of glucagon action with decreasing concentrations of SST.

From the fact that SST inhibits the secretions of several hormones such as GH, insulin, glucagon and thyroid-stimulating hormone and since the presence of Ca is essential for normal secretory processes of various hormones, Curry and Bennett (1974) showed that this inhibitory action of SST involves antagonism or activation of Ca and that an elevation of concentration of Ca above physiological limits reversed the SST inhibition of hormonal secretion by perfused rat pancreas. Based on studies in the perfused unrecirculated rat pancreas, Bathena et al (1976) postulated a mechanism of SST action which is shown on Figure 4. According to this mechanism, SST acts on the islets of Langerhans to impair Ca^{++} ion uptake. In the absence of Ca^{++}, neither glucagon nor insulin secretion occurs, the essential role of Ca^{++} ion presumably entailing

(a) generation of c-AMP and (b) requirements for the secretory process distal to c-AMP action.

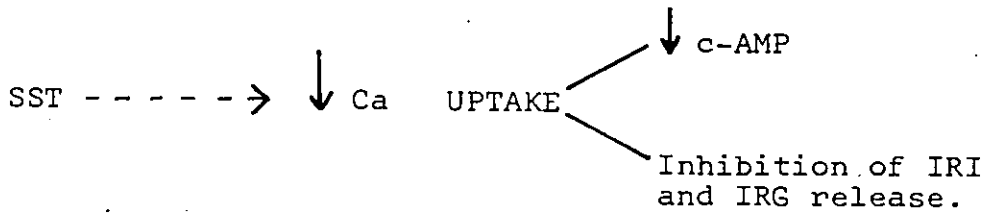


Figure 4: Schematic representation of the inhibitory effect of somatostatin(SST) on insulin(IRI) and glucagon(IRG) secretion.(Bathena et al,1976)

In 1977, electrophysiological studies of rat islets cells were undertaken to determine the role of transmembrane ionic fluxes in the inhibitory action of SST on insulin release (Pace et all, 1977). Increasing extracellular concentration of K^+ or Ca^{++} prevented SST inhibition of glucose-induced electrical activity. It was concluded that the primary action of SST is to alter transmembranous cationic fluxes, which may prevent glucose from eliciting a normal secretory response.

CHAPTER III

MATERIALS AND METHODS

Animals

All experiments were performed on 12 non-anesthetized normal healthy mongrel dogs ranging between 8.2 and 19.1 kg, unselected for sex. The dogs were trained to stand quietly in a Pavlov stand. All animals were in the post-absorptive state having been fasted sixteen to twenty hours after their last meal. The diet of the animals contained 49% protein, 8.5% fat, 32% carbohydrate and 16.5% fibre on a dry weight basis. This diet was supplemented for one to two days after each experiment with meatballs containing 10.6% protein and 14.7% fat. Food and water consumption was ad libitum.

Procedures

Two plastic catheters (Clay Adams PE 190 or 205) were inserted under local procaine anaesthesia approximately fifteen to thirty minutes before the experiment, one into the cephalic vein and the other into the inferior vena cava via the saphenous vein. All infusions were given via the first catheter, priming doses of ^{3}H-3-glucose and somatostatin were given and blood samples were taken via

the second catheter.

Blood samples were transfered immediately after withdrawal into pre-cooled plastic centrifuge tubes containing dry heparin, mixed and kept in crushed ice until centrifugation. One ml of whole blood was preserved in Trasylol:EDTA (1:1) solution, mixed, centrifuged at 2,000 revolutions per minute (r.p.m.) for ten minutes, the hematocrit was measured and the plasma saved for glucagon determination, being placed at -2 to -4°C within five minutes. If the plasma was to be analyzed for FFA, not more than five minutes were allowed to elapse between the withdrawal and the centrifugation of blood. No more than fifteen to twenty minutes elapsed between the sampling and centrifugation for ten minutes at 2,000 r.p.m. Plasma for cold and ^{3}H-3-glucose determinations as well as for the insulin determinations was immediately frozen as such at -2 to -4°C. Free fatty acid determinations were performed on the day of the experiment, starting with the addition of 50 λ of plasma to a mixture of silicic acid and chloroform-heptane-methanol (28:21:1).

Experimental Design

Three series of experiments were carried out. In the first series each dog was subjected to two experiments. In

one a 100 minutes long infusion of saline (phase 1) was followed by a 70 minutes infusion of either 50 μg/kg.min phlorizin or 7 mU/kg.min insulin (phase 2). This phase was followed by a 70 minutes infusion of phlorizin or insulin with 0.35 μg/kg.min somatostatin added to the infusion (phase 3). At the start of phase 3, at t = 170 minutes, 10 μg/kg somatostatin was injected. A final 40 minutes period during which saline was infused finished the experiment (phase 4).

Each dog was submitted to a pair of experiments: in one experiment insulin, in the other phlorizin was infused. The sequence of the two experiments on the same dog was at random. Altogether 5 dogs were tested in 10 experiments.

The design in the second series differed from that used in the first only in that sequence of phase 2 and phase 3 was reversed. The 100 minutes infusion of saline was followed by an infusion of phlorizin or insulin plus somatostatin, and this in turn by the infusion of insulin or phlorizin alone. Altogether three dogs were tested in this way in six experiments.

All experiments in the third series consisted of three phases. After an infusion of saline for 100 minutes, 7 mU/kg.min insulin was infused for 70 minutes. This in turn was followed by another infusion of saline for another

100 minutes. In another experiment on the same animal 0.35 μg/kg.min somatostatin was also infused during the second and third phases (i.e from t = 100 to t = 270 min). Altogether 8 experiments were carried out on 4 dogs.

Timing of Blood Samples

In the experiments in the first and second series, 21 samples were taken at t = 8, 15, 30, 50, 65, 80, 90, 100, 110, 125, 140, 155, 170, 180, 195, 210, 225, 240, 250, 265 and 280 minutes. In the experiments in the third series, 20 samples were taken at an identical schedule until t = 240 minutes and after this at t = 255 and 270 minutes. Glucose and radioactive glucose were determined in all samples. Plasma free fatty acid concentration was determined at t = 65, 80, 100, 125, 170, 195, 240, 250 and 280 minutes in experiments of the first and second series at the same times in experiments of the third series until t = 240 minutes after which one more sample was analyzed at t = 270 minutes. Immunoreactive insulin and glucagon was determined at the same points of time as FFA except at t = 65 minutes.

Chemicals

^{3}H-3-glucose was purchased from New England Nuclear

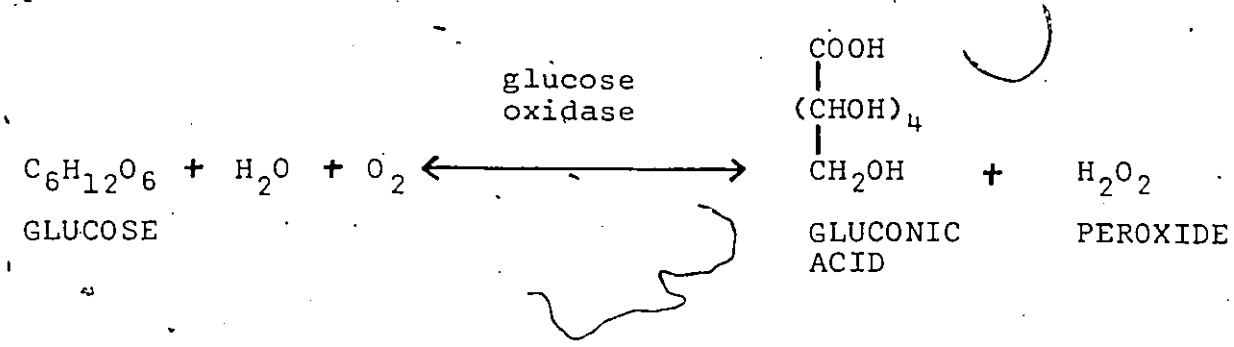
(Boston MA, USA). A kit produced from the Amersham Corporation (Oakville, Ontario, Canada) was used for the determination of insulin. Somatostatin was a generous gift of the Ayerst Research Laboratories (Montreal, P.Q.,Canada). All chemicals were obtained from Fisher Scientific Co. (Toronto, Ont., Canada) and were of the highest grade of purity available.

Determination of the Concentration of Metabolites and Hormones in the Plasma

a. The Determination of Glucose in the Plasma (C, mg/dl)

To remove non-sugar reducing substances along with proteins, the plasma was first deproteinized with $Ba(OH)_2$ and $ZnSO_4$ according to the method described by Somogyi (1945).

Plasma glucose was determined enzymatically as described by Huggett and Nixon (1957). This method (Bergmeyer and Bernt, 1965) is based on the specific oxidation of glucose by glucose oxidase. Oxygen is released from the resulting peroxide by peroxidase in the presence of the chromogenic oxygen acceptor o-dianisidine. This coupled oxidation proceeds as shown here:



$$H_2O_2 + \text{Dye-}H_2 \xrightarrow{\text{peroxidase}} \text{Dye(coloured)} + 2\,H_2O$$

PEROXIDE O-DIANISIDINE

After 60 minutes of incubation at 37°C, maximum chromogenesis had taken place, and the samples were read at 420 Å on a spectrophotometer (Coleman III). A quality control test has been done and is given in the Appendix II and on Table 25.

b. The Determination of the Concentration of ^{3}H-3-glucose in the Plasma (C^*, dpm/ml)

The concentration of labeled glucose in each sample was determined in terms of disintegrations per minute (dpm) of ionizing β radiations emitted by the isotope ^{3}H. To achieve this, one ml of deproteinized plasma filtrate was evaporated at 80°C in vacuo and the dry residue was re-dissolved in one ml of distilled water. Ten ml of Bray's scintillator solution was added and, after a cooling period of 15 minutes, the mixture was counted in a Liquid Scintillation Spectrometer (Hetenyi and Mak, 1970).

c. The Determination of Free Fatty Acids in the Plasma (FFA, pg/ml)

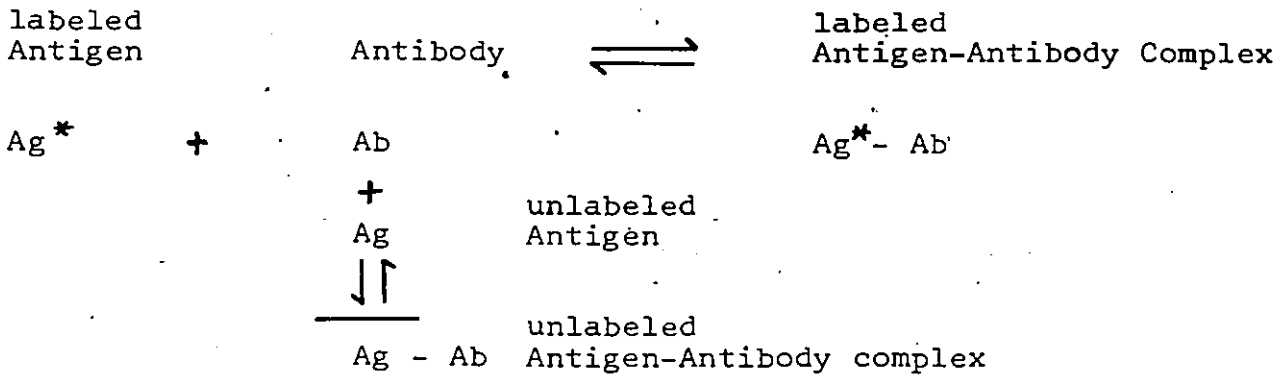
Free fatty acids were determined by a colorimetric

micromethod based on the formation of FFA-Cu soaps, as described by Laurell and Tibbling (1967). To eliminate the possible interference by phospholipids, silicic acid was added to the extraction fluid. FFAs were extracted with chloroform to which heptane (4:3 v/v) and 2% methanol were added. Heptane and saturation of CuTEA reagent with sodium chloride were used to obtain the CuTEA reagent as the lower phase. Methanol was added for an optimal FFA recovery. Saponification of FFA was achieved with CuTEA reagent. Diphenylcarbazide was used for the colorimetric determination of copper. Centrifugation at low temperature as well as special care in choosing stopcocks were necessary to avoid waste of the volatile extracting solution along the determination. Standards and unknowns were read at 550 Å on a spectrophotometer (Coleman III).The results were calculated from a standard curve for two different concentrations of palmitic acid. Determinations were made in duplicate for the standards and each sample. A quality control test has been done and is given in the Appendix II and on Table 26.

d. The Determination of Insulin in the Plasma (IRI, μU/ml)

A kit manufactured for the purpose of determining plasma immunoreactive insulin (IRI) concentrations was

purchased from Amersham-Searle. The methodology used to measure insulin is called competitive radioimmunoassay and the reactions involved are summarized below:



Basically, the unlabeled antigen(Ag) competes with the labeled antigen (Ag^*) for a limited number of antibody (Ab) binding sites and decreases the amount of labeled antigen bound to the antibody (Ag^* - Ab). If the amounts of the antibody and labeled antigen are the same for all samples, the only variable is the amount of unlabeled antigen. Therefore unknown samples can be quantitated by comparing the decrease in antibody binding of labeled antigen in unknowns and in known standard solutions. In order to separate the labeled antigen-antibody complex from the free labeled antigen, a modification of the double antibody method of Hales and Randle (1963) was used.

Standard solutions of insulin and samples were first added to the double antibody (insulin binding) reagent and incubated for six hours at 2-4°C. Radioactive insulin (^{125}I-insulin) was then added and the mixture incubated for another eighteen hours at the same temperature. This is the so-called 24 hour assay. For the measurement of concentrations of insulin smaller than 16 μU/ml, a modification of this assay was used. It is the 96 hour insulin assay which differs mainly in the length of incubation and the dilution of reactants, but which is basically the same procedure. Thus, in order to separate the insoluble labeled antigen-antibody complex from the soluble labeled free insulin, both the standards and samples were washed with 4% bovine serum albumin (fraction V) buffer and filtered through millipore membranes (0.45 μm) using a millipore apparatus. The membranes were carefully dried in a vacuum oven, cooled at room temperature and 10 ml of Bray's scintillation fluid was added. The radioactivity was counted, after 15 minutes of cooling, on a Liquid Scintillation Spectrometer set for the ^{3}H energy spectrum.

The counting of ^{125}I-insulin is possible at this setting for the following reason. The gamma-rays emitted by the isotope ^{125}I are in fact particles of very high energies undetectable as such by the Scintillation Counter

functioning as a beta-spectrometer. However, what is described as "Compton scattering or effect" occurs. That is a collision between two particles, an incident particle or photon (gamma ray) and an atomic electron to which this photon imparts some of its energy (Young, H.D., 1976). The result: an outgoing photon having less energy and smaller frequency than the incident one, and an atomic electron having enough energy to be removed from orbit and become a high-speed electron with the same energy level as the beta-particle. This therefore can be detected by the Scintillation Spectrometer (coincidentally at the setting used for the detection of ^{3}H). A quality control test has been done and is given in the Appendix II and on Table 27.

e. The Determination of Glucagon (IRG, pg/ml) and Glucagon-like Immunoreactivity (GLI, pg/ml) in the Plasma.

Plasma samples were collected in a solution of Trasylol (500 units/ml of blood) to protect them against the proteolytic loss of glucagon during collection and preparation of the plasma samples (Heding, 1969). Sodium EDTA (24 mg/ml) was used as the anticoagulant. The samples were kept in the deep freeze (-10 to -20°C) and transported packed with dry ice. Glucagon (IRG) was determined by an immunoassay

using specific antibody 30K (Faloona and Unger, 1974).

Glucagon-like immunoreactivity (GLI) was also determined for certain experiments by an immunoassay using non-specific antiserum K-4023 and by substracting the values of glucagon (IRG) from that obtained with the non-specific antiserum, as read against a glucagon standard curve (Muller et al, 1978). Determinations and calculations for IRG and GLI were kindly done by Dr Mladen Vranic and his laboratory assistants at the University of Toronto.

Calculations

a. Rates of Appearance and Disappearance of Glucose

Calculation of the rates of appearance (Ra), identical with the hepatic glucose production, and disappearance (Rd), identical with the overall glucose utilization plus glucosuria, was with the primed tracer infusion method originally described by De Bodo et al (1963). This method assumes a one compartment glucose system having different mechanisms controlling the production rate (input) by the liver and its disappearance (output) in the various tissues of the body. The rate of appearance of glucose is calculated as

$$\frac{Ra}{Kg} = \left[\left(F - pV\,\frac{C_1 + C_2}{2} \times \frac{SA_2 - SA_1}{T_2 - T_1}\right) \times \frac{1}{(1/2)(SA_1 + SA_2)}\right]\left[\frac{1}{Kg}\right]$$

where Ra is the rate of appearance of glucose (mg/kg-min), F is the infusion rate of labeled glucose (5.55×10^5 d.p.m/min) p is the "pool fraction" taken to be equal to 0.65 (Cowan and Hetenyi, 1971; Radziuk et al, 1978), V is the glucose distribution volume (ml) calculated from the intercept of the priming injection of tracer. V was taken to be equal to $Q \times CP_1 / CTRL \times SA_0$ where Q is the volume of tracer glucose injected (0.25 ml), CP_1 is the concentration of the injected tracer (dpm/ml), CTRL is the basal glucose concentration taken as the average glucose concentration of the four last samples of the control period (mg/ml), SA_0 is the specific activity (C^*/C, dpm/mg) of glucose at time 0. This value was calculated by linear regression. C_1 and C_2 are plasma glucose concentrations (mg/ml) and SA_1 and SA_2 are the specific activities at times T_1 and T_2 (dpm/mg). The rate of disappearance of glucose is calculated as

$$\frac{Rd}{Kg} = \left[\frac{Ra}{Kg}^{(Kg)} - pV \quad \frac{C_2 - C_1}{T_2 - T_1}\right]\left[\frac{1}{Kg}\right]$$

where Rd is the rate of disappearance of glucose (mg/kg-min) and the other symbols are the same as explained above. All calculations were carried out on a Wang 600 desk calculator.

b. Statistical Analysis

A paired t-test was used to assess the statistical significance of an increment in any two selected measured or calculated quantities. When a change in some quantity was compared between two phases within an experiment, a complete analysis of variance was carried out with the original data and the F and P values calculated for the "difference between phases" was taken as a measure of significance (Snedecor, 1957). A similar analysis of variance was used to assess the differences between plasma glucose, FFA and IRI samples with respect to the variation between parallel determinations carried out on the same plasma sample. This test was used as a procedure for the "quality control" of the determinations. It was carried out with the results of 3 randomly selected experiments.

A special analysis of variance was used to analyze the results of the third series of experiments. For this purpose the BMDP -2V program was used on an IBM 360/65 computer. Variation between treatment (somatostatin infused vs control dogs), animals, points of time in the experiment and interactions between the variables were considered (See Appendix I, Table 22 to 24).

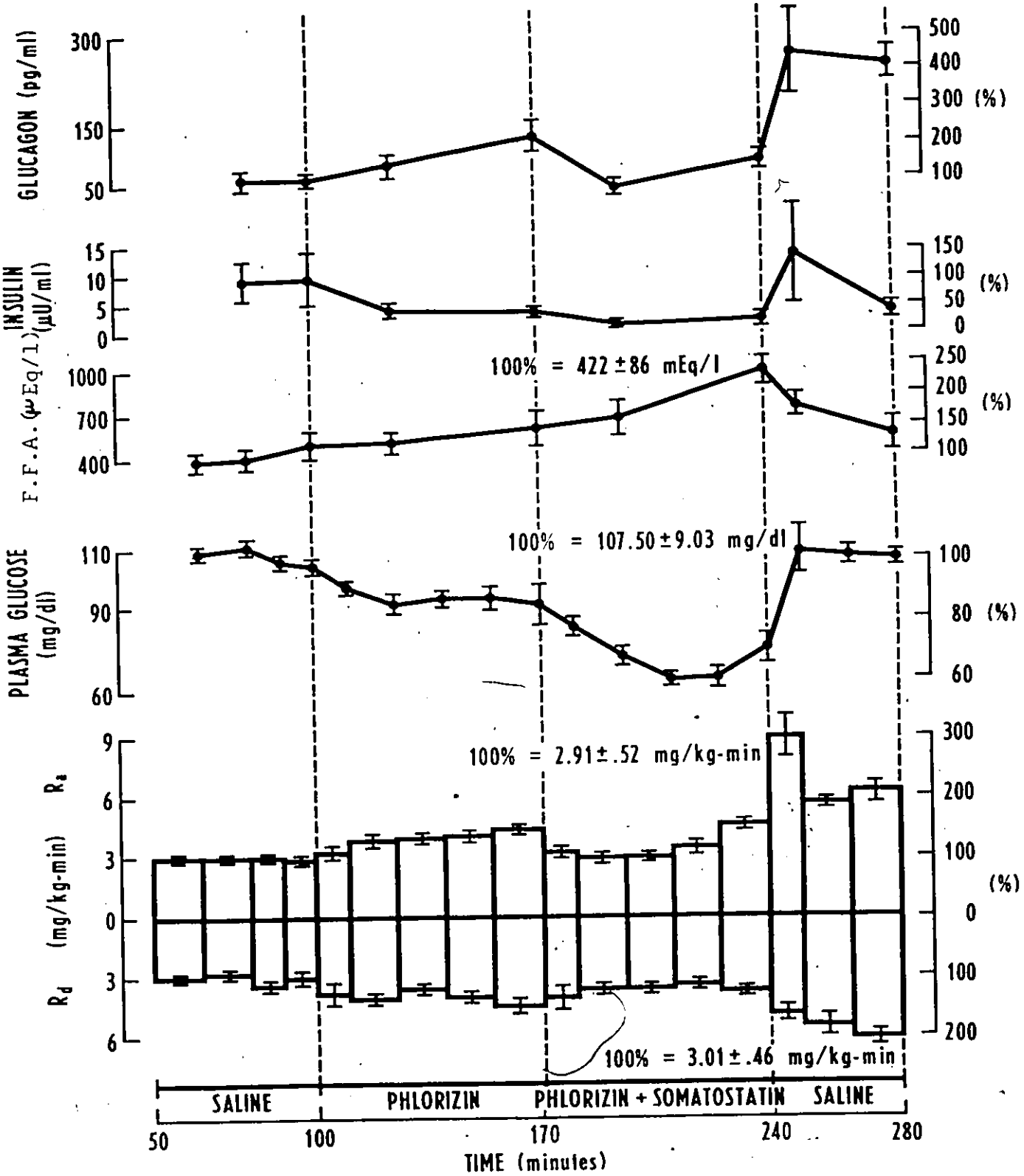
CHAPTER IV

RESULTS

The results of the first series of experiments are shown on Figures 5 and 6. It is evident from Figure 5 that the infusion of phlorizin caused a small decrease of about 15% in the concentration of plasma glucose. During this time, the rate of glucose production was increased compared to that observed during the first phase ($F = 32.51$, $P < 0.005$). The concentration of IRG in the plasma was significantly higher than during the control phase ($F = 14.73$, $P < 0.01$), whereas the concentration of IRI was somewhat lower ($F = 3.51$, $P > 0.05$). This decrease in plasma IRI however was not statistically significant. There was no significant change in the concentration of plasma FFA. The rate of disappearance of glucose (i.e. the rate of removal of glucose from the circulation) was increased by approximately 1 mg/kg.min. The addition of somatostatin to phlorizin in the infusion (phase 3) resulted in the marked diminution in the concentration of plasma glucose, simultaneously with a decrease in glucose production. The decrease between the rate of glucose production in the second phase and the first 40 minutes of the third phase was statistically significant ($F = 17.71$, $P < 0.005$). In the latter half of phase 3, the rate of glucose production increased slowly. This blunted the difference

Figure 5: The effect of an infusion of phlorizin (50 µg/kg.min.) followed by that of phlorizin plus somatostatin (0.35 µg/kg.min.) on the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on glucose appearance (R_a) and disappearance (R_d). Abscissa: time in minutes. Ordinates on the left show the variables in absolute units. On the ordinates on the right the variables are expressed as the percentage of their control values. Standard errors of means are shown as vertical bars. For Ra, Rd and the plasma concentrations of glucose and FFA they refer to the percentage value, for IRI and IRG to absolute concentrations. Mean values of 5 experiments are shown.

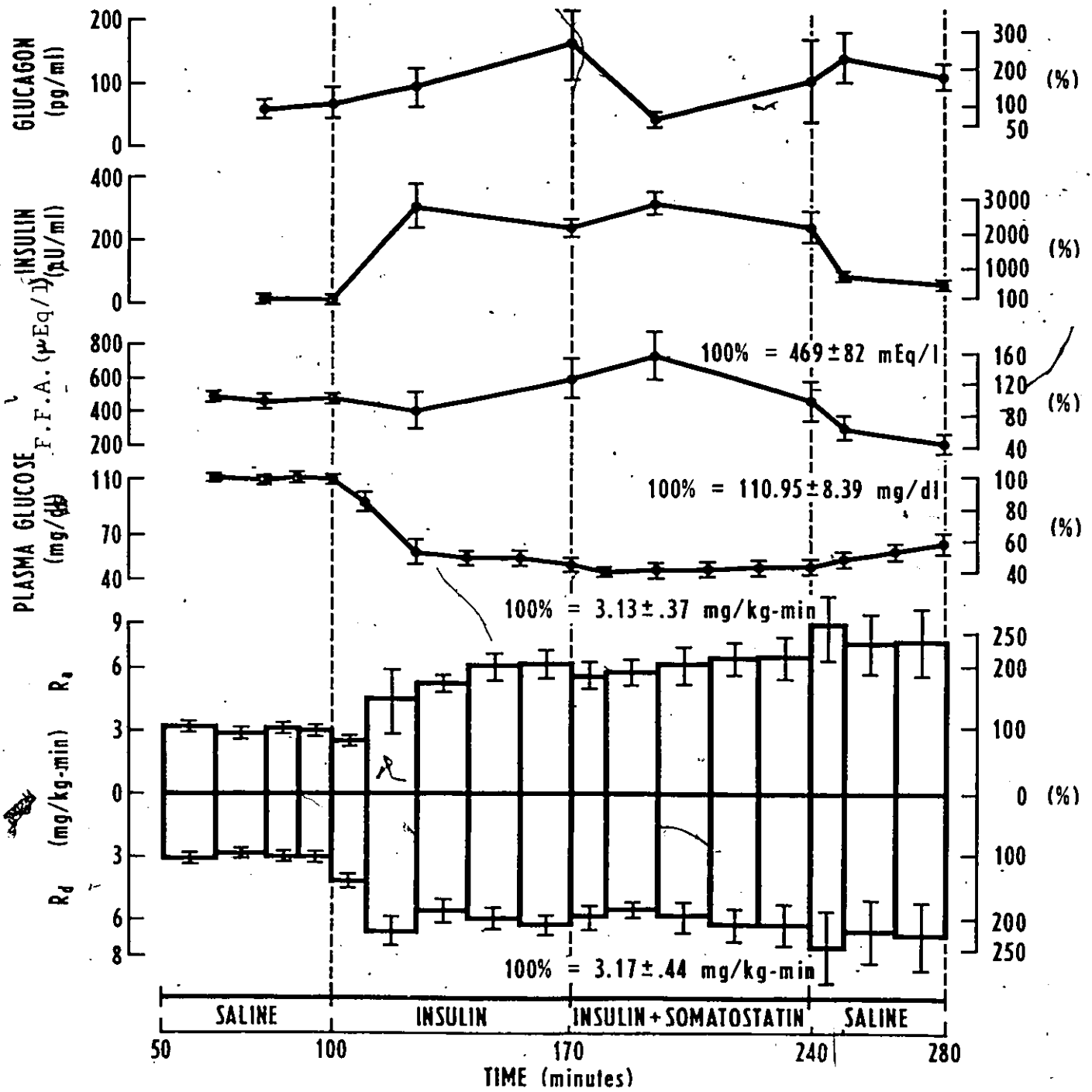
Figure 5


GLUCAGON (pg/ml)
300
150
50
500
400
300 (%)
200
100
INSULIN (μU/ml)
15
10
5
0
150
100
50
0
(%)
F.F.A. (μEq/l)
1000
700
400
100% = 422 ± 86 mEq/l
250
200
150
100
(%)
PLASMA GLUCOSE (mg/dl)
110
90
60
100% = 107.50 ± 9.03 mg/dl
100
80
60
(%)
R_a (mg/kg-min)
R_d
9
6
3
0
3
6
100% = 2.91 ± .52 mg/kg-min
300
200
100
0
100
200
(%)
100% = 3.01 ± .46 mg/kg-min
SALINE
PHLORIZIN
PHLORIZIN + SOMATOSTATIN
SALINE
50
100
170
240
280
TIME (minutes)

between the two phases. The addition of somatostatin to the infusion of phlorizin led to a decrease in the concentration of plasma IRG ($F = 10.76$, $P < 0.01$), the concentration of IRI remained below its basal (phase 1) level ($F = 5.64$, $P < 0.05$). The concentration of plasma FFA did not change significantly until time = 240 minutes, when the concentration of glucose fell below 70 mg/dl. At this time it was increased significantly above the control (time = 100 min) level ($t = 6.50$, $p < 0.01$). The increase during the third phase (between 170 and 240 min.) was also significant ($t = 3.99$, $p < 0.025$). When the infusion of phlorizin plus somatostatin was discontinued, both the rate of glucose production and the concentration of plasma glucose rose within 10 minutes. Both increments are statistically significant ($t = 3.42$, $p < 0.05$ and $t = 4.21$, $p < 0.025$ respectively). These changes were synchronous with a sudden rise in plasma IRG ($t = 2.66$, $p \lesssim 0.05$). The rise in the concentration of IRG in the plasma was sustained during this phase ($F = 54.15$, $P < 0.005$). A transient rise in plasma IRI between time 240 and 250 minutes was observed in three of the five animals. The statistical analysis of the results of these experiments is given in the Appendix I and on Tables 2 to 6.

Figure 6: The effect of an infusion of insulin (7 mU/kg.min.) followed by that of insulin plus somatostatin (0.35 µg/kg.min.) on the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on glucose appearance (Ra) and disappearance (Rd). Mean values of 5 experiments are shown. See the legend to Figure 5 for details.

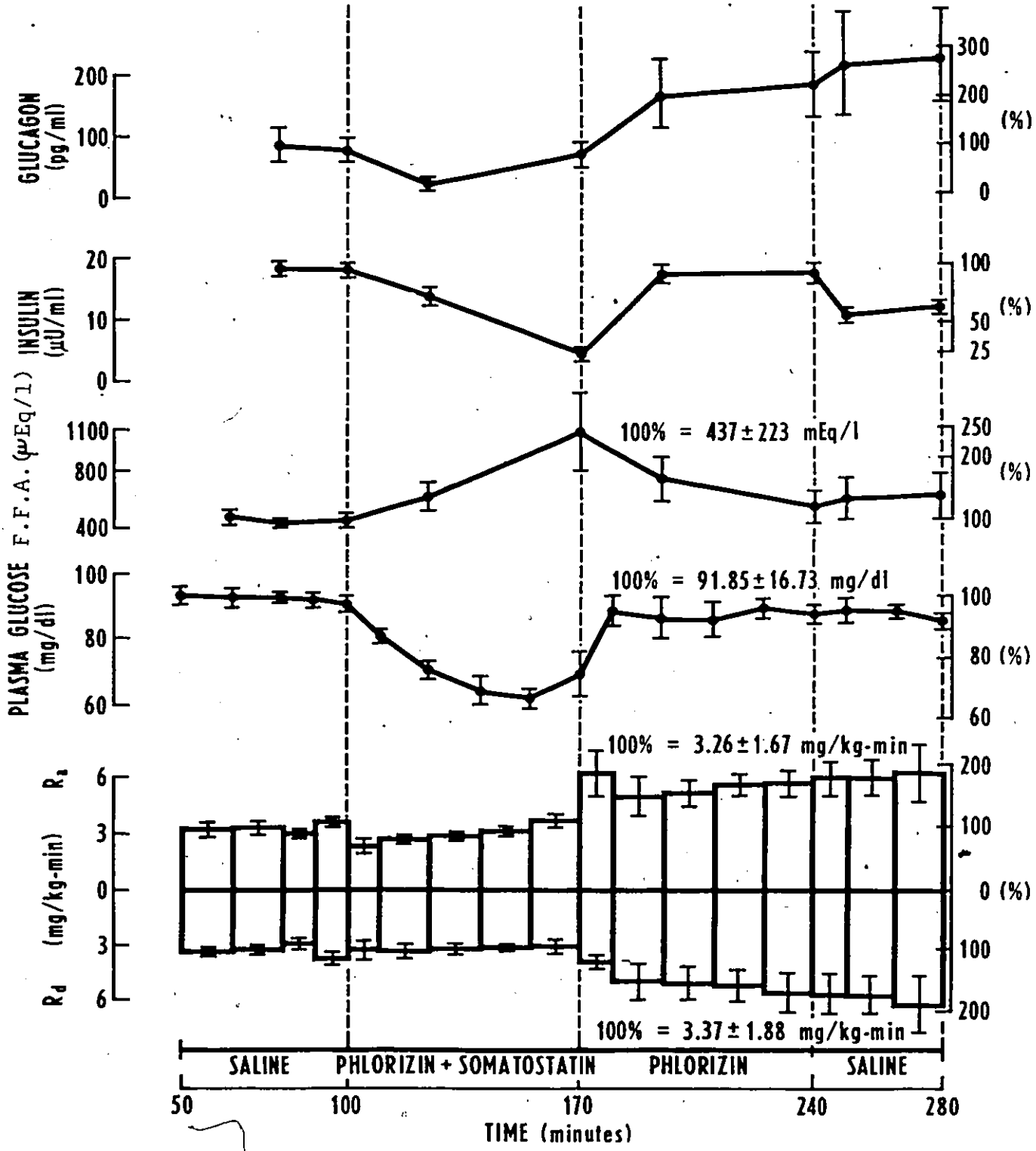
Figure 6


GLUCAGON (pg/ml)
INSULIN (μU/ml)
F.F.A. (μEq/l)
PLASMA GLUCOSE (mg/dl)
100% = 469±82 mEq/l
100% = 110.95±8.39 mg/dl
100% = 3.13±.37 mg/kg-min
100% = 3.17±.44 mg/kg-min
(%)
SALINE
INSULIN
INSULIN+SOMATOSTATIN
SALINE
50
100
170
240
280
TIME (minutes)

On Figure 6 the effects of insulin induced hypoglycemia are shown. Insulin reduced the level of glucose to a mean of 48 ± 3 mg/dl. This hypoglycemia brought forth an increased release of glucose into the circulation. The difference between the basal Ra and the Ra observed during steady hypoglycemia (after time = 125 min.) is significant ($F = 183.7$, $P < 0.005$). There was also a significant increase in IRG during this phase ($F = 8.19$, $P < 0.025$). The addition of somatostatin to insulin in the infusion in the third phase did not changed significantly the rate of glucose production although the level of plasma IRG was suddenly reduced (the increment between plasma IRG at time 170 and 195 min. is significant, $t = 2.68, p < 0.05$). The concentration of IRG in the plasma however did not remain consistently at a level below that observed in the third phase ($F = 3.28$, $P > 0.05$). Plasma FFA concentration was not changed significantly in spite of an elevated level of IRI in the plasma. When the infusion of insulin plus somatostatin was turned off no significant change in Ra was observed. Both plasma IRG and IRI concentrations were above their basal level (observed during phase 1) during this final phase ($F = 39.00$, $P < 0.005$ for IRI; $F = 26.46$, $P < 0.005$ for IRG). The rate of disappearance of glucose, which in the absence of glycosuria equals the rate of glucose utilization, was still elevated.

Figure 7: The effect of an infusion of phlorizin (50 μg/kg.min.) plus somatostatin (0.35 μg/kg.min.) followed by that of phlorizin on the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on the glucose appearance (Ra) and disappearance (Rd). Mean values of 3 experiments are shown. See the legend to Figure 5 for details.

Figure 7

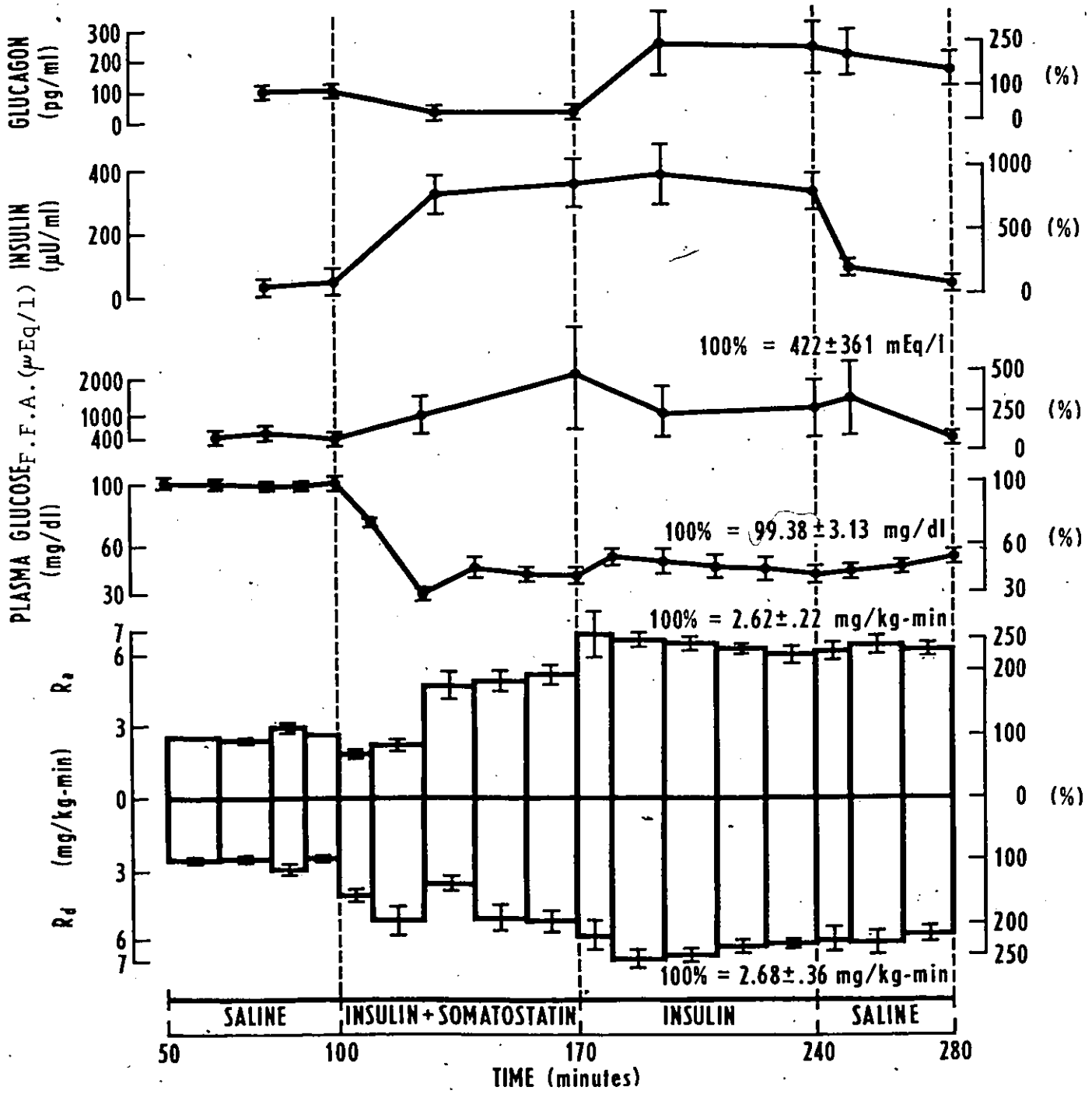

GLUCAGON (pg/ml)
200
100
0
300
200
100
0
(%)
INSULIN (μU/ml)
20
10
0
100
50
25
(%)
F.F.A. (μEq/l)
1100
800
400
100% = 437 ± 223 mEq/l
250
200
100
(%)
PLASMA GLUCOSE (mg/dl)
100
80
60
100% = 91.85 ± 16.73 mg/dl
100
80
60
(%)
100% = 3.26 ± 1.67 mg/kg-min
R_a
R_d (mg/kg-min)
6
3
0
3
6
200
100
0
100
200
(%)
100% = 3.37 ± 1.88 mg/kg-min
SALINE
PHLORIZIN + SOMATOSTATIN
PHLORIZIN
SALINE
50
100
170
240
280
TIME (minutes)

The statistical analysis of the results of these experiments is given in the Appendix I and on Tables 7 to 10.

The rationale of the second series of experiments was to ascertain whether the decreases in the concentration of IRG in the plasma and in the rate of glucose production remain parallel if the sequence of the infusions of phlorizin or insulin without or with somatostatin is reversed. As shown in Figure 7 during the simultaneous infusion of phlorizin and somatostatin the rate of glucose production does not change significantly as compared to the control phase ($F = 3.19$, $P < 0.1$) in spite of the low levels in plasma IRI and glucose observed in this phase. There was a significant rise in plasma FFA ($F = 15.04$, $P < 0.005$) and a slight, but statistically non significant ($F = 4.45$, $P < 0.1$) depression in the concentration of plasma IRI. When the infusion of somatostatin was discontinued and phlorizin only was infused, the rate of glucose production was increased ($F = 85.96$, $P < 0.005$). There was also a significant increase in the concentration of plasma glucose ($F = 96.83$, $P < 0.005$), in spite of the return of plasma IRI to its basal level. The concentration of plasma IRG was significantly increased ($F = 56.86$, $P < 0.005$). The decline in the level of FFA in the plasma was indicated but not statistically significant ($F = 2.14$, $P > 0.1$). The statistical analysis of the results

Figure 8: The effect of an infusion of insulin (7 mU/kg.min.) plus somatostatin followed by that of insulin on the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on the glucose appearance (Ra) and disappearance (Rd). Mean values of 3 experiments are shown. See the legend to Figure 5 for details.

Figure 8

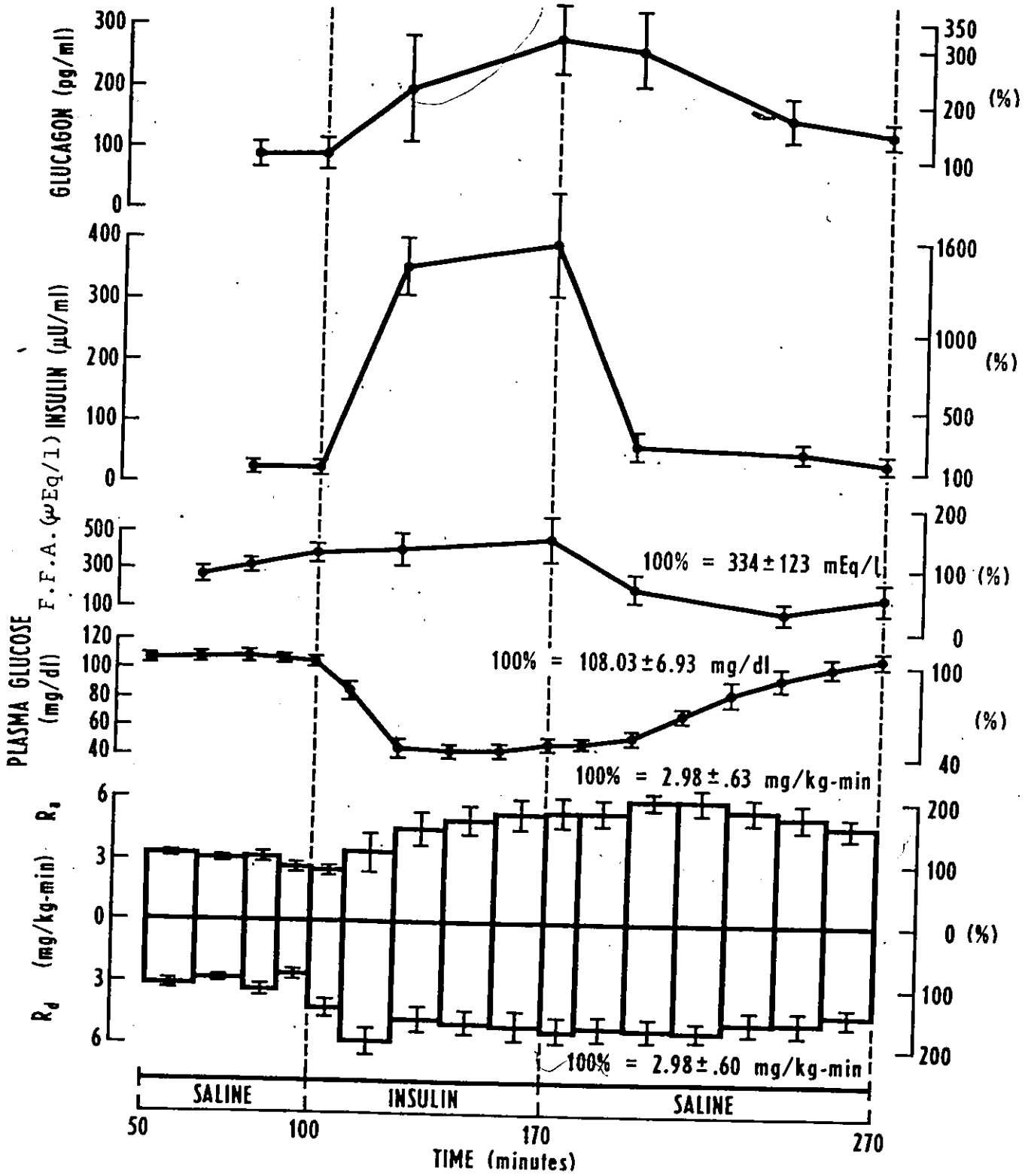

GLUCAGON (pg/ml)
300
200
100
0
250
100
0
(%)
INSULIN (μU/ml)
400
200
0
1000
500
0
(%)
100% = 422±361 mEq/l
F.F.A. (μEq/l)
2000
1000
400
500
250
0
(%)
PLASMA GLUCOSE (mg/dl)
100
60
30
100% = 99.38±3.13 mg/dl
100
60
30
(%)
100% = 2.62±.22 mg/kg-min
Ra
Rd (mg/kg-min)
7
6
3
0
3
6
7
250
200
100
0
100
200
250
(%)
100% = 2.68±.36 mg/kg-min
SALINE
INSULIN + SOMATOSTATIN
INSULIN
SALINE
50
100
170
240
280
TIME (minutes)

of these experiments is given in the Appendix I and on Tables 11 to 15.

Results of similar experiments carried out with insulin instead of phlorizin are shown on Figure 8. During the infusion of insulin plus somatostatin the rate of glucose production rose well above its basal level once hypoglycemia was established (i.e. after time = 125 min.). The difference was significant ($F = 226.7$, $P < 0.005$). The concentration of plasma IRG was decreased below its basal level ($F = 133.7$, $P < 0.005$). When the infusion of insulin plus somatostatin was changed to that of insulin alone, the rate of glucose production increased. The difference in glucose production between the last 55 minutes of the second phase and that in the third is significant ($F = 31.69$, $P < 0.005$). The concentration of IRG in the plasma was also increased ($F = 191.2$, $P < 0.005$). The discontinuation of somatostatin added to the infusion of insulin had no significant effects on either the concentration of glucose or on the elevated level of FFA in the plasma. When the infusion of insulin too was discontinued (at time = 240 min.), no significant change did occur in any other measured parameter than the level of plasma IRI during the 40 minute period of observation (phase 4). The statistical analysis of the results of these experiments is given in the Appendix I and on Tables 16 and 17.

Figure 9: Changes in the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on the glucose appearance (Ra) and disappearance (Rd) during and after the infusion of 7 mU/kg.min. insulin. Mean values of 4 experiments are shown. See the legend to Figure 5 for details.

Figure 9

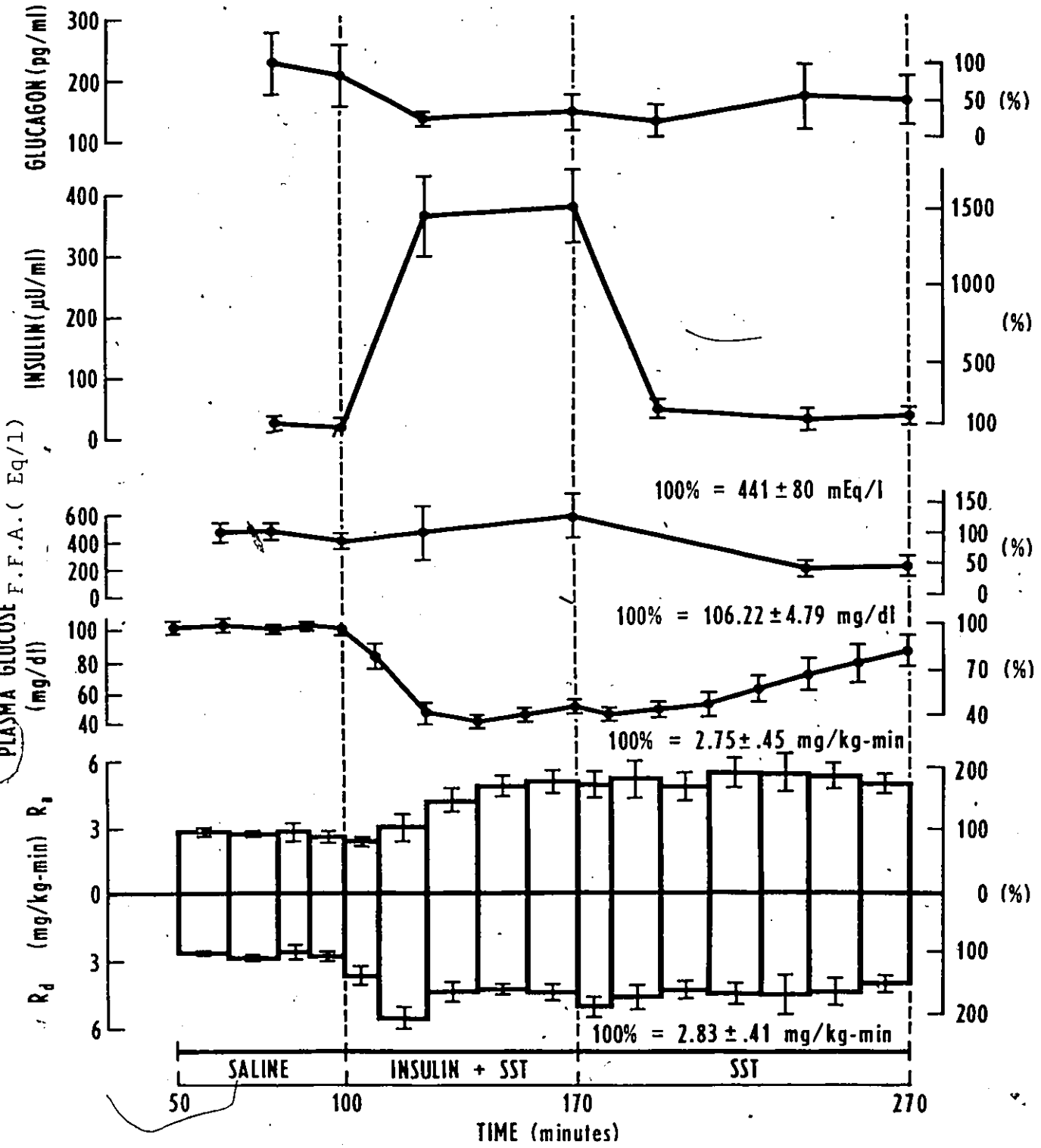

GLUCAGON (pg/ml)
300
200
100
0
350
300
200
100
(%)
INSULIN (μU/ml)
400
300
200
100
0
1600
1000
500
100
(%)
F.F.A. (μEq/l)
500
300
100
100% = 334±123 mEq/l
200
100
(%)
0
PLASMA GLUCOSE (mg/dl)
120
100
80
60
40
100% = 108.03±6.93 mg/dl
100
(%)
40
100% = 2.98±.63 mg/kg-min
R_a (mg/kg-min)
R_d
6
3
0
3
6
200
100
0 (%)
100
200
100% = 2.98±.60 mg/kg-min
SALINE
INSULIN
SALINE
50
100
170
270
TIME (minutes)

The aim of the third series of experiments was to examine the effect of somatostatin on the speed of recovery from insulin-induced hypoglycemia. Figure 9 shows the results of control experiments in which 7 mU/kg.min. was infused for 70 minutes and the speed of recovery followed over a period of 100 minutes. Thirty minutes after cessation of the infusion of insulin the concentration of glucose in the plasma began to rise, because the rate of glucose production exceeded the rate of utilization by about 25%. Plasma IRG concentration rose during the infusion of insulin ($F = 26.11$, $P < 0.005$). In the recovery phase it followed a parallel course to glucose production. There was no significant change in plasma FFA concentration during the infusion of insulin. When the infusion was stopped the concentration of FFA dropped below its basal (first phase) level ($F = 25.04$, $P < 0.005$). The statistical analysis of the results of these experiments is given in the Appendix I and on Tables 18 and 19.

When somatostatin was infused together with insulin and also after the infusion of insulin was stopped (Figure 10), the rise in the concentration of IRG was prevented, and the concentration of plasma IRG decreased to around 45 pg/ml ($F = 13.49$, $P < 0.005$). In spite of this the rate of glucose production was increased during the infusion of insulin

Figure 10: Changes in the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on the glucose appearance (Ra) and disappearance (Rd) during the infusion of insulin (7 mU/kg.min.) plus somatostatin (SST: 0.35 µg/kg.min.) and during a recovery period from hypoglycaemia when somatostatin alone was infused. Mean values of 4 experiments are shown. See legend to Figure 5 for details.

Figure 10


GLUCAGON (pg/ml)
300
200
100
100
50 (%)
0
INSULIN (μU/ml)
400
300
200
100
0
1500
1000
(%)
500
100
100% = 441 ± 80 mEq/l
F.F.A. (Eq/l)
600
400
200
0
150
100
50 (%)
0
100% = 106.22 ± 4.79 mg/dl
PLASMA GLUCOSE (mg/dl)
100
80
60
40
100
70 (%)
40
100% = 2.75 ± .45 mg/kg-min
Ra (mg/kg-min) Rd
6
3
0
3
6
200
100
0 (%)
100
200
100% = 2.83 ± .41 mg/kg-min
SALINE
INSULIN + SST
SST
50
100
170
270
TIME (minutes)

and remained high during the recovery period. The rate of glucose production was only marginally lower in dogs infused with somatostatin. This difference was not significant statistically. The speed at which the concentration of plasma glucose approached the normal range after the cessation of infusion of insulin however was significantly slower in dogs to which somatostatin was infused ($F = 18.34$, $P < 0.01$). The statistical analysis of the results of these experiments is given in the Appendix I and on Tables 20 and 21. Results shown on Tables 22 to 24 have been calculated by Dr S. Raman of the Royal Ottawa Hospital. There was no significant difference between the course of the plasma concentration vs time curves of FFA in somatostatin infused and control animals.

Glucagon-like immunoreactivity (GLI) in plasma was also determined in the dogs in third series of experiments. The results are shown on Figure 11 and Figure 12. By comparing these two figures with Figure 9 and Figure 10, it can be seen that the extrahepatic glucagon shows the same general trend than pancreatic glucagon. In order to show the parallel behabior of both types of glucagon during an insulin and insulin-somatostatin infusion, the ratio of plasma glucagon concentration (IRG) to glucagon-like immunoreactivity (GLI) was calculated and the data are reported on Table 1.

Figure 11 : Changes in the plasma concentration of glucagon-like immunoreactivity (GLI) and glucose during and after the infusion of 7 mU/kg.min. insulin. Mean values of 4 experiments are shown. See the legend to Figure 5 for details.

Figure 12 : Changes in the plasma concentration of glucagon-like immunoreactivity (GLI) and glucose during the infusion of insulin (7 mU/kg.min.) plus somatostatin (SST: 0.35 μg/kg.min) and during a recovery period from hypoglycaemia when somatostatin alone was infused. Mean values of 4 experiments are shown. See legend to figure 5 for details.

Figure 11

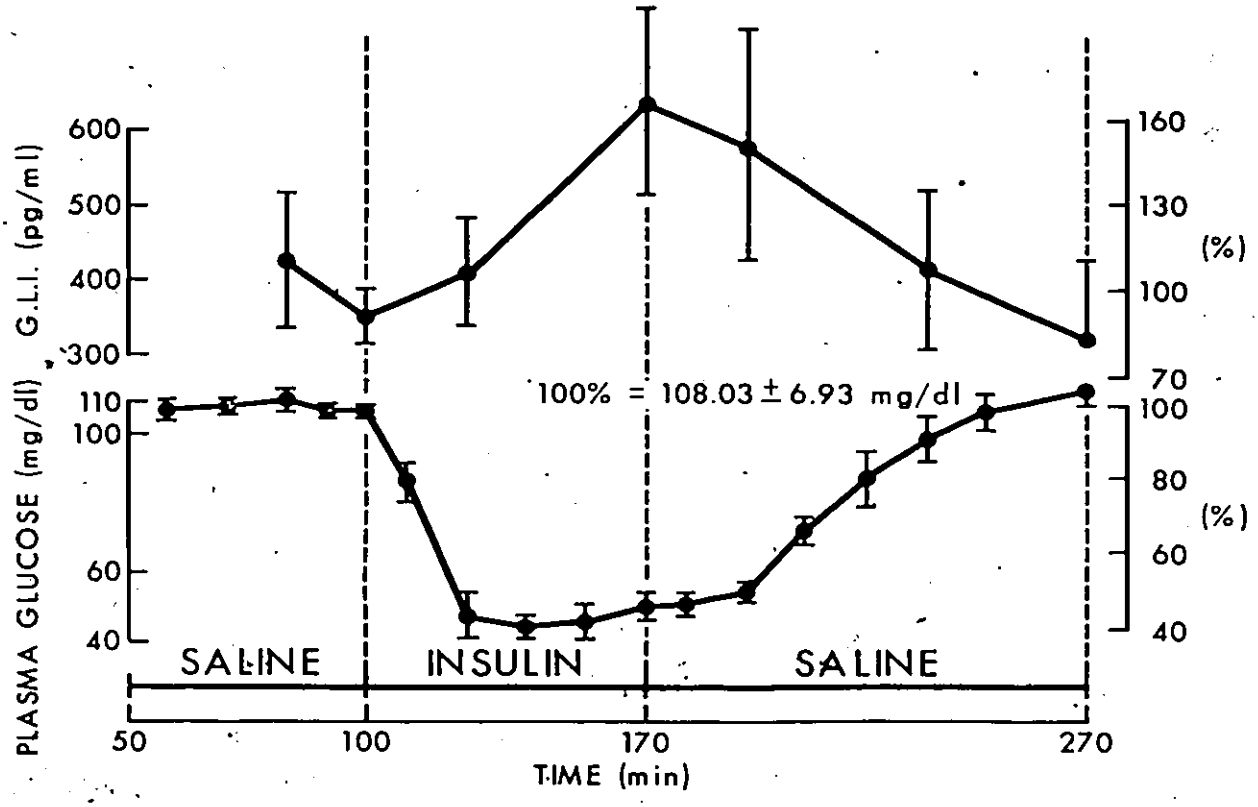


Figure 12

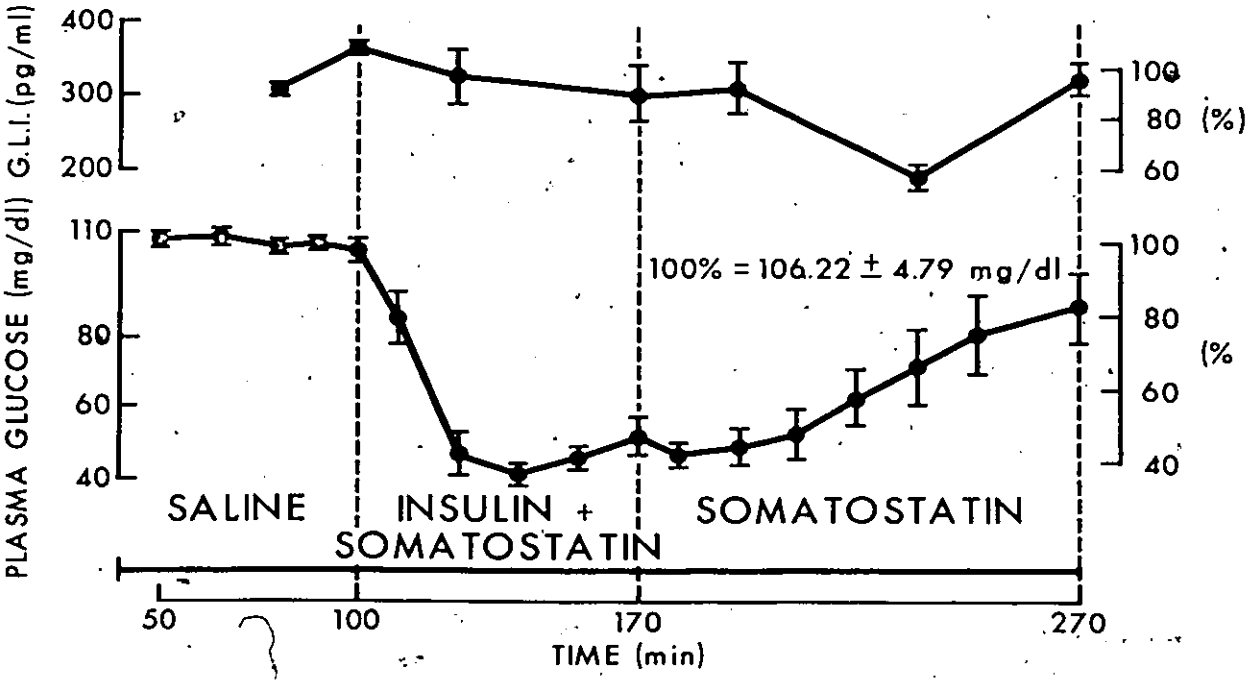


TABLE 1

RATIO OF PLASMA GLUCAGON CONCENTRATION (IRG) TO GLUCAGON-LIKE IMMUNOREACTIVITY (GLI) IN INSULIN-INFUSED POST-ABSORPTIVE DOGS WITH AND WITHOUT SOMATOSTATIN.

Treatment	IRG/GLI AT DIFFERENT TIMES (min.)						
	80	100	125	170	195	240	270
without somatostatin	.210 ± .211	.258 ± .115	.485 ± .250	.446 ± .191	.461 ± .246	.378 ± .233	.410 ± .246
with somatostatin	.426 ± .103	.301 ± .329	.127 ± .098	.158 ± .172	.106 ± .163	.360 ± .227	.189 ± .144

Note: Ratio of mean values and S.E.M. are shown. n = 3

CHAPTER V

DISCUSSION

The experiments presented clearly demonstrate that an increased loss of glucose from the plasma into the urine to a modest degree, estimated to be approximately 1 mg/kg.min caused by the infusion of phlorizin leads to an approximately equivalent increase in the rate of hepatic glucose production as previously observed (Kolodny et al, 1962). As a result of this increase, the concentration of glucose in the plasma is only marginally reduced. This compensatory increase in hepatic glucose production appears to be brought about by an increase in the concentration of glucagon in the plasma, since the concentration of plasma insulin did not change during the infusion of phlorizin. If the rise in the concentration of glucagon is prevented by the simultaneous infusion of somatostatin, the increase in the rate of glucose release is abolished. During the simultaneous infusion of phlorizin and somatostatin the concentration of glucose in plasma drops to about 65 mg/dl. The infusion of 0.35 μg/kg.min somatostatin was shown to induce a decrease of only about 15% of the basal glucose level in conscious dogs (Lickley et al, 1979). The experiments presented offer the answer to the first two of the four

questions posed in the Introduction. In vivo, the α-cells seem to be very sensitive even to a marginal decrease in the level of plasma glucose. Consequently the regulation of plasma glucagon concentration is very precise. This control is achieved by glucagon. Therefore we conclude that one important physiological function of glucagon is in the maintenance of plasma glucose at around the set point in face of an increased rate of disappearance of glucose from the plasma. This agrees with the concept of glucagon being an important regulator of glucose production in the dog in the post-absorptive and fasting state (Altszuler et al, 1976; Bergman, 1977; Lickley et al, 1979). Similar results are also obtained in exercising dogs (Issekutz and Vranic, 1978). Normoglycemia was maintained in face of a three-fold increase of glucose utilization when glucagon rose and increments of glucose production matched the increased fuel demand of the working muscle. Plasma glucose decreased sharply however, when somatostatin was infused during exercise, plasma glucagon was suppressed and therefore glucose production did not increased adequately.

As shown on Figure 5, the concentration of FFA in the plasma was significantly increased ($t = 3.99$, $p < 0.025$) when the concentration of glucose fell to about 65 mg/dl. Since neither IRI or IRG changed significantly between

195 and 240 minutes, the very significant increase in plasma FFA ($t = 10.47$, $p < .001$) is possibly due to the release of cathecolamines at this low level of plasma glucose (De Fronzo et al, 1977; Goldfien et al, 1958). The low level of IRI might have accentuated this effect of epinephrine.

The last phase of the experiment shown on Figure 5 deserves some attention. Ten minutes after the cessation of the phlorizin and somatostatin infusions, the IRG level increased to 4 times of its basal value and remained there for the rest of the recovery phase, even though the plasma glucose concentration returned to its basal level. Due to the fact that the IRG concentration increased only by a factor of 2 even after 70 minutes of continuous infusion of phlorizin during the second phase, delayed phlorizin effect could hardly be responsible for this high IRG level, still elevated 40 minutes after the cessation of the phlorizin and somatostatin infusion. A possible explanation of this phenomenon could be a mechanism similar to the so-called "memory effect" observed in pancreatic islets from glucose-infused rats in relation with insulin secretion (Cole and Logothetopoulos, 1974). A better understanding of this phenomenon could have been obtained had the recovery from phlorizin and somatostatin been followed longer.

A different picture is seen in overt, insulin induced hypoglycemia when the concentration of plasma glucose has been reduced to the range of 40-45 mg/dl. The rate of hepatic glucose production increases ($F = 183.69$, $P < .005$) to an even greater extent than during the infusion of phlorizin ($F = 32.52$, $P < .005$). However even this increase is unable to restore normoglycemia in the presence of the high level of plasma IRI concentration and the consequent marked increase in the rate of glucose utilization. The concentration of glucagon in plasma rises in insulin induced hypoglycemia. The infusion of somatostatin again prevents this rise in plasma IRG, but with only little, if any effect on the increase in the rate of hepatic glucose production. Evidently glucagon is either not a significant factor in augmenting the rate of hepatic glucose release in hypoglycemia, or if the increase in the secretion of glucagon is prevented, other factors are mobilized to increase the release of glucose into the circulation (Gerich et al, 1979). The fact that this factor cannot be blocked by somatostatin suggests catecholamines (Chideckel et al, 1975), which are known to be released in large amounts during marked (De Fronzo et al,1977; Goldfien et al,1958; Wallace and Harlan,1965) but not marginal (Christensen et al,1975; De Fronzo et al,1977) hypoglycemia in the resting

state. The modest, statistically not significant increase in the concentration of plasma FFA in the presence of an extremely high plasma IRI level during insulin induced hypoglycemia yields some support to this assumption.

The usefulness of plasma FFA as a reliable index of the release of catecholamines is severely curtailed by any change in the level of IRI in the plasma. Should this increase, the inhibitory effect of insulin on lipolysis will mask the effect of catecholamines. Conversely a decrease in plasma IRI may by itself increase the FFA concentration in the plasma. It is furthermore likely that as the level of glucose in the plasma decreases, the release of glucose from the liver increases because of an increase in glycogenolysis. This response is not hormone mediated (Bergman, 1977; Sacca et al, 1979). the concentration of glucose itself is the regulator. The release of glucose by this mechanism is inversely related to the concentration of glucose in the plasma thus much more glucose is released in overt hypoglycemia than during the infusion of phlorizin near the basal blood glucose level.

The results of experiments presented therefore indicate that glucagon is a <u>regulator</u> of plasma glucose around the set point, whereas other mechanisms including

the release of a significant amount of glucose by epinephrine or by autoregulation (Bergman, 1977) are more likely emergency mechanisms activated when this finer regulation breaks down and the concentration of glucose in the plasma becomes as low as to be unable to supply an adequate amount of glucose for the function of the brain (Hetenyi, 1972). However, as the increase in the rate of glucose production at time 170 minutes on Figure 8 demonstrates, a surge in the release of glucagon when superimposed on an increased adrenergic drive may still cause an appreciable further increase in hepatic glucose production. At a basal (normal) level of plasma glucose the effect of catecholamines on the rate of glucose production was found to be transient (Gray et al, 1978). When however epinephrine was infused together with glucagon and cortisol a synergism between their hyperglycemic effects was observed: the ensuing hyperglycemia being more marked and prolonged than the sum of the individual effects of these hormones (Eigler et al, 1979). The role of glucagon in the homeostatic mechanisms activated by hypoglycemia may be in this potentiation of the effects of catecho lamines and perhaps even cortisol (Eigler et al, 1979), although neither cortisol (Feldman et al, 1975; Gerich et al, 1979) nor epinephrine (Walter et al, 1974) in itself play an indispensable

role in the process of recovery from hypoglycemia caused by a single injection of insulin in man. The relative importance of the role of glucagon in this process in dogs is reflected in the speed at which normoglycemia is restored after the cessation of an infusion of insulin. The comparison of Figures 9 and 10 reveals that a normal glucose level in plasma is attained more slowly if somatostatin is infused during and after insulin ($F = 18.34$, $P < 0.01$).

As shown on Figures 11 and 12, the extrapancreatic glucagon-like activity was mobilized by hypoglycemia to an even greater extent than pancreatic glucagon. However, by comparing with Figures 9 and 10, it can be seen that both types of glucagon react similarly during insulin recovery with and without somatostatin. This agrees with other similarities between the immunologic and biologic effects of pancreatic and extrapancreatic glucagon (Vranic et al, 1977). The large standard errors of the means shown on Table 1 are in good part due to the fact that GLI values were calculated as the difference of two determinations by immunoassay: one specific for IRG the other measuring the sum of IRG and GLI. Therefore if the ratio IRG/GLI is calculated the errors in the determination are compounded.

In summary, the results of our experiments reveal two mechanisms maintaining plasma glucose level and an adequate supply of glucose to all tissues: glucagon is a true physiological regulator which maintains plasma glucose with good precision at, or very near its set point. Once this sensitive and precise regulatory mechanism is broken down by extreme rates of disappearance of glucose from the plasma, other factors may be called into play as emergency mechanisms.

CHAPTER VI

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CHAPTER VII

APPENDIX I

STATISTICAL ANALYSIS OF RESULTS

TABLE 2

RATE OF APPEARANCE OF GLUCOSE (Ra) IN PHLORIZIN AND PHLORIZIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($50 \leq t \leq 100$) vs Phase 2 ($110 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	7.937	7.937	32.517
				$P < 0.005$
between dogs	4	10.753	2.688	11.013
				$P < 0.005$
interaction	4	0.385	0.096	0.393
error	35	8.543	0.244	

B. Phase 2 ($110 \leq t \leq 170$) vs Phase 3 ($180 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.648	1.648	3.483
				$P < 0.1$
between dogs	4	16.852	4.213	8.901
				$P < 0.05$
interaction	4	0.341	0.085	
error	40	18.932	0.473	

C. Phase 2 ($110 \leq t \leq 170$) vs Phase 3 ($180 \leq t \leq 210$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	5.131	5.131	17.717
				$P < 0.005$
between dogs	4	10.767	2.691	9.293
				$P < 0.005$
interaction	4	0.064	0.016	0.055
error	30	8.689	0.289	

TABLE 3

CONCENTRATION OF PLASMA GLUCAGON (IRG) IN PHLORIZIN AND PHLORIZIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	11045	11045	14.734 $P < 0.01$
between dogs	4	12910	3222	4.305 $P < 0.025$
interaction	4	2132	533	0.711
error	10	7496	749	

B. Phase 2 ($125 \leq t \leq 170$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	11776	11776	10.757 $P < 0.01$
between dogs	4	7466	1866	1.705
interaction	4	6517	1629	1.488
error	9	9852	1094	

C. Phase 3 ($195 \leq t \leq 240$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	187129	187129	54.148 $P < 0.005$
between dogs	4	28058	7014	2.029
interaction	4	36664	9166	2.65
error	9	31102	3455	

TABLE 4

CONCENTRATION OF PLASMA INSULIN (IRI) IN PHLORIZIN AND PHLORIZIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	146.34	146.34	3.511
between dogs	4	62.53	15.63	0.375
interaction	4	72.37	18.09	0.434
error	10	416.71	41.67	

B. Phase 2 ($125 \leq t \leq 170$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	20.82	20.82	8.625 $P < 0.025$
between dogs	4	7.46	1.87	0.773
interaction	4	6.31	1.58	0.653
error	9	21.72	2.41	

C. Phase 3 ($195 \leq t \leq 240$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	197.99	197.99	2.217
between dogs	4	491.82	122.96	1.376
interaction	4	480.53	120.13	1.345
error	9	803.72	89.30	

TABLE 4 (continuation)

D. Phase 1 ($80 \leq t \leq 100$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	259.1	259.1	5.635 $P < 0.05$
between dogs	4	57.93	14.48	0.315
interaction	4	56.6	14.15	0.307
error	9	413.77	45.97	

TABLE 5

CHANGE IN CONCENTRATION OF PLASMA FREE FATTY ACIDS (FFA) IN PHLORIZIN AND PHLORIZIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS. PAIRED STUDENT'S t TEST

Experiment	Δ FFA($t_{170}-t_{100}$)	Δ FFA($t_{240}-t_{100}$)	Δ FFA($t_{240}-t_{170}$)
1	+ 120	585	465
2	+ 285	675	158
3	− 120	420	530
4	+ 65	385	210
5	+ 190	878	723
Mean	108	588.6	417.2
S.E.M.	67.83	89.75	104.55
t	1.592	6.558	3.990
P		< 0.01	< 0.025

TABLE 6 a

CHANGE IN THE CONCENTRATION OF PLASMA GLACAGON (IRG), INSULIN (IRI) AND FREE FATTY ACIDS (FFA) BETWEEN 195 AND 240 MINUTES IN PHLORIZIN AND PHLORIZIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS.

PAIRED STUDENT'S t TEST

Experiment	Δ IRG	Δ IRI	Δ FFA
1	42	- .48	.328
2	23	—	.145
3	-12	.32	.450
4	58	- .20	.220
5	—	4.30	.533
Mean	27.75	.98	.335
S.E.M.	15.06	1.12	.032
t	1.84	.88	10.47
P			$< .001$

TABLE 6 b

CHANGE IN THE RATE OF GLUCOSE APPEARANCE(Ra), AND CONCENTRATION OF PLASMA GLUCOSE(G),GLUCAGON(IRG) AND INSULIN(IRI) BETWEEN 240 and 250 MINUTES IN PHLORIZIN AND PHLORIZIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS.

PAIRED STUDENT'S t TEST

Experiment	Δ Ra	Δ C	Δ IRG	Δ IRI
1	8180	61.4	427	46.9
2	3.52	25.5	47	8.14
3	5.03	38.9	149	2.62
4	4.17	36.7	116	- 0.2
5	0.74	11.3	133	0.0
Mean	4.452	34.76	174.4	11.49
S.E.M.	1.303	8.266	65.5	8.98
t	3.417	4.205	2.663	1.28
p	< 0.05	< 0.025	≈ 0.05	

TABLE 7

RATE OF APPEARANCE OF GLUCOSE (Ra) IN INSULIN AND INSULIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($50 \leq t \leq 100$) vs Phase 2 ($140 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	72.193	72.193	183.69 $P < 0.005$
between dogs	4	15.626	3.906	9.939 $P < 0.005$
interaction	4	9.134	2.283	5.809 $P < 0.01$
error	25	9.826	0.393	

TABLE 8

CONCENTRATION OF PLASMA GLUCAGON (IRG) IN INSULIN AND INSULIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	22656	22656	8.189 $P < 0.025$
between dogs	4	31927	7981	2.885
interaction	4	25330	6332	2.289
error	9	24898	2766	

B. Phase 2 ($125 \leq t \leq 170$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	18734	18734	3.278
between dogs	4	80593	20148	3.526
interaction	4	2980	745	0.13
error	9	51424	5713	

C. Phase 3 ($195 \leq t \leq 240$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	20465	20465	5.28 $P < 0.05$
between dogs	4	46859	11714	3.02
interaction	4	3485	871	0.224
error	9	34878	3875	

TABLE 8 (continuation)

D. Phase 1 ($80 \leq t \leq 100$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	24555	24555	26.46 $P < 0.005$
between dogs	4	16192	4048	4.36
interaction	4	7835	1958	2.11
error	9	8352	928	

TABLE 9

CONCENTRATION OF PLASMA INSULIN (IRI) IN INSULIN AND INSULIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($80 \leq t \leq 100$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	17117	17117	39.0 $P < 0.005$
between dogs	4	187.1	46.8	0.106
interaction	4	416.9	104.2	0.237
error	10	4388.9	438.9	

TABLE 10

CHANGE IN CONCENTRATION OF PLASMA FREE FATTY ACIDS (FFA) IN INSULIN AND INSULIN-SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS

PAIRED STUDENT'S t TEST

Experiment	Δ FFA ($t_{170}-t_{100}$)	Δ FFA ($t_{240}-t_{100}$)	Δ FFA ($t_{195}-t_{100}$)	Δ FFA ($t_{240}-t_{170}$)
6	233	135	438	-102
7	287	-215	90	-255
8	282	295	465	13
9	-195	-200	- 90	- 5
10	375	50	500	-325
Mean	196.4	13.0	280.6	-134.8
S.E.M.	100.4	98.27	118.45	67.23
t	1.95	0.13	2.36	-2.76
p				

TABLE 11

RATE OF APPEARANCE OF GLUCOSE (Ra) IN PHLORIZIN-SOMATOSTATIN AND PHLORIZIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($50 \leq t \leq 100$) vs Phase 2 ($110 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	0.951	0.951	3.188
between dogs	2	43.438	21.719	72.759 $P < 0.005$
interaction	2	0.482	0.241	0.807
error	21	6.268	0.298	

B. Phase 2 ($110 \leq t \leq 170$) vs Phase 3 ($180 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	33.033	33.033	85.966 $P < 0.005$
between dogs	2	34.538	17.269	44.942 $P < 0.005$
interaction	2	0.509	0.254	0.663
error	24	9.222	0.384	

TABLE 12

CONCENTRATION OF PLASMA GLUCAGON (IRG) IN PHLORIZIN-SOMATOSTATIN AND PHLORIZIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	4218.7	4218.7	5.19
between dogs	2	3508.2	1754.1	2.16
interaction	2	1981.5	990.8	1.22
error	6	4876.5	812.8	

B. Phase 2 ($125 \leq t \leq 170$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	46750.1	46750.1	56.87 $P < 0.005$
between dogs	2	23355.5	11677.8	14.2 $P < 0.01$
interaction	2	13322.2	6661.1	8.1 $P < 0.05$
error	6	4932.5	822.1	

C. Phase 3 ($195 \leq t \leq 240$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	6210.7	6210.8	25.41 $P < 0.005$
between dogs	2	102454.2	51227.1	209.59 $P < 0.005$
interaction	2	7275.5	3637.8	14.88 $P < 0.005$
error	6	1466.5	244.4	

TABLE 13

CONCENTRATION OF PLASMA INSULIN (IRI) IN PHLORIZIN-SOMATOSTATIN AND PHLORIZIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	280.33	280.33	4.447
between dogs	2	759.79	379.89	6.027 $P < 0.05$
interaction	2	17.16	8.58	0.136
error	6	378.16	63.03	

B. Phase 2 ($125 \leq t \leq 170$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	210.84	210.84	3.2
between dogs	2	1767.71	883.85	13.4 $P < 0.01$
interaction	2	204.25	102.12	1.6
error	6	395.26	65.88	

C. Phase 3 ($195 \leq t \leq 240$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	132.67	132.67	13.95 $P < 0.05$
between dogs	2	891.70	445.85	46.88 $P < 0.005$
interaction	2	858.89	429.44	45.16 $P < 0.005$
error	6	57.06	9.51	

TABLE 14

CONCENTRATION OF PLASMA GLUCOSE (C) IN PHLORIZIN-SOMATOSTATIN AND PHLORIZIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

Phase 2 ($140 \leq t \leq 170$) vs Phase 3 ($180 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2425.49	2425.49	96.825 $P < 0.005$
between dogs	2	3753.7	1876.9	74.916 $P < 0.005$
interaction	2	297.68	148.8	5.941 $P < 0.05$
error	18	450.95	25.05	

TABLE 15

CONCENTRATION OF PLASMA FREE FATTY ACIDS (FFA) IN PHLORIZIN-SOMATOSTATIN AND PHLORIZIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($65 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	330633	330633	15.037 $P < 0.005$
between dogs	2	390123	195061	8.871 $P < 0.01$
interaction	2	102577	51288	2.332
error	9	197879	21986	

B. Phase 2 ($125 \leq t \leq 170$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	84168	84168	2.135
between dogs	2	314654	157327	3.99
interaction	2	17712	8866	0.224
error	6	236437	39406	

TABLE 16

RATE OF APPEARANCE OF GLUCOSE (Ra) IN INSULIN-SOMATOSTATIN AND INSULIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($50 \leq t \leq 100$) vs Phase 2 ($140 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	28.207	28.207	226.74 $P < 0.005$
between dogs	2	4.256	2.128	17.11 $P < 0.005$
interaction	2	1.52	0.760	6.11 $P < 0.05$
error	15	1.866	0.124	

B. Phase 2 ($140 \leq t \leq 170$) vs Phase 3 ($180 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	12.473	12.473	31.694 $P < 0.005$
between dogs	2	12.209	6.104	15.512 $P < 0.005$
interaction	2	0.118	0.059	0.149
error	18	7.083	0.393	

TABLE 17

CONCENTRATION OF PLASMA GLUCAGON (IRG) IN INSULIN-SOMATOSTATIN AND INSULIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	15577.79	15577.7	133.714 $P < 0.005$
between dogs	2	24.43	12.2	0.104
interaction	2	1699.86	849.9	7.295 $P < 0.05$
error	5	582.5	116.5	

B. Phase 2 ($125 \leq t \leq 170$) vs Phase 3 ($195 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	135894.1	135894.1	191.242 $P < 0.005$
between dogs	2	50984	25492	35.874 $P < 0.005$
interaction	2	44192	22096.3	31.096 $P < 0.005$
error	6	4263.5	710.6	

C. Phase 3 ($195 \leq t \leq 240$) vs Phase 4 ($250 \leq t \leq 280$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	10034.1	10034.1	6.143 $P < 0.05$
between dogs	2	121016	60508	37.047 $P < 0.005$
interaction	2	7424.7	3712.3	2.272
error	6	9799.5	1633	

TABLE 18

CONCENTRATION OF PLASMA GLUCAGON (IRG) IN INSULIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	69312.00	69312.00	26.114 $P < 0.005$
between dogs	2	40758.17	20379.02	7.678 $P < 0.05$
interaction	2	15571.5	7785.75	2.933
error	6	15925.00	2654.17	

TABLE 19

CONCENTRATION OF PLASMA FREE FATTY ACIDS (FFA) IN INSULIN-INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($80 \leq t \leq 100$) vs Phase 3 ($195 \leq t \leq 270$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	181656	181656	25.04 $P < 0.005$
between dogs	3	149043	49681	6.85 $P < 0.001$
interaction	3	54084	18028	2.48
error	16	116036	7252	

TABLE 20

CONCENTRATION OF PLASMA GLUCAGON (IRG) IN INSULIN-SOMATOSTATIN AND SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($80 \leq t \leq 100$) vs Phase 2 ($125 \leq t \leq 170$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	11656.33	11656.33	7.705 $P < 0.05$
between dogs	2	10458.5	5229.25	3.455
interaction	2	1643.17	821.58	0.542
error	6	9081.00	1513.50	

B. Phase 1 ($80 \leq t \leq 100$) vs Phase 3 ($195 \leq t \leq 270$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	9985.60	9985.60	7.748 $P < 0.025$
between dogs	2	29488.47	14744.23	11.441 $P < 0.01$
interaction	2	326.87	163.43	0.126
error	9	11598.33	1288.7	

TABLE 21

CONCENTRATION OF PLASMA FREE FATTY ACIDS (FFA) IN INSULIN-SOMATOSTATIN AND SOMATOSTATIN INFUSED POST-ABSORPTIVE DOGS

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($80 \leq t \leq 100$) vs Phase 3 ($195 \leq t \leq 270$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	228540	228540	15.42 $P < 0.005$
between dogs	3	29161	9720	.656
interaction	3	97593	32531	2.19
error	16	236996	14812	

TABLE 22

RATE OF APPEARANCE OF GLUCOSE (Ra) IN INSULIN INFUSED POST-ABSORPTIVE DOGS WITH AND WITHOUT SOMATOSTATIN ($180 \leq t \leq 270$)

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between treatment	1	2.044	2.044	2.42 $.2 < P < .1$
between animals	3	7.083	2.361	2.79
time (Total)	6	3.995	.666	< 1
linear trend	1	.295	.295	< 1
residual	5	3.700	.740	< 1
interaction				
Time x Treatment	6	2.500	.417	
Time x Animals	18	13.152	.731	
error	21	17.744	.845	

TABLE 23

RATE OF DISAPPEARANCE OF GLUCOSE (Rd) IN INSULIN INFUSED POST-ABSORPTIVE DOGS WITH AND WITHOUT SOMATOSTATIN ($180 \leq t \leq 270$)

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between treatment	1	.530	.530	1.02
between animals	3	3.861	1.287	2.17
time (Total)	6	3.749	.625	1.05
linear trend	1	.668	.668	1.12
residual	5	3.081	.616	1.03
interaction				
Time x Treatment	6	.582	.097	
Time x Animals	18	10.694	.594	
error	21	10.939	.521	

TABLE 24

CONCENTRATION OF PLASMA GLUCOSE (C) IN INSULIN INFUSED POST-ABSORPTIVE DOGS WITH AND WITHOUT SOMATOSTATIN ($180 \leq t \leq 270$)

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
treatment	1	4670.8	4670.8	18.34 $P < 0.01$
animals	3	6033.8	2011.3	13.92 $P < 0.01$
time (Total)	7	24088	3441.2	13.51 $P < 0.01$
linear trend	1	22885	22885	31.56 $P < 0.001$
residual	6	1202.8	200.5	< 1
interaction				
Time x Treatment	7	1782.6	254.7	5.01 $P < 0.01$
Time x Animals	21	3034.4	144.5	2.84 $P < 0.01$
error	24	1220.8	50.87	

As shown on Tables 24,22 and 23, the high statistical significance in the glucose concentrations in the absence of significance of both glucose production and utilization may at the outset appear paradoxical. The explanation may be sought in the measurement protocols. The rates of utilization and production are obtained as the arithmetic means of two points measurements spaced over an interval of 10 or 15 minutes. Consequently these mean values do not represent the dynamics of the rates that take place during this interval of time. The magnitude of the noise component precludes the collection of more information by more frequent sampling. Consequently the absence of significance in the production and utilization rates in these experiments reflect the poor resolution of the measurement process, rather than an intrinsic absence of treatment effect. As shown on Table 24 the difference between the time courses of plasma glucose concentration was highly significant between control and somatostatin treated animals. The variation among animals and also over the time intervals were very highly significant. Further the interaction components with time were also highly significant. The statistical significance over time could be explained entirely by the linear trend (i.e. a steadily increasing concentration).

APPENDIX II

QUALITY CONTROL

A. Counting Error

Radioactive counting is a manifestation of isolated events occuring within a specific time interval which follows the distribution law of Poisson. The mean value (x) of a Poisson's distribution being the same than its variance, the standard error of the mean (S.E.M.) will thus be the square root of this mean value (Moroney, 1970). The counting error will thus be equal to $\sqrt{x}$.

^{3}H-3-glucose

The total number of counts registered over a period of 20 minutes was between 2,000 to 20,000 counts for ^{3}H-3-glucose, so that the counting error was between 2% and 0.7% depending on the number of registered counts.

^{125}I-insulin

(a) 24 hour insulin assay:

The counts were between 12,000 and 40,000 over a period of 4 minutes, so that the counting error is between 0.9% and 0.5% for the 24 hour insulin assay.

(b) 96 hour insulin assay:

The counts were between 2,800 and 8,000, so that

the counting error is between 1.8% and 1.2% for the 96 hour insulin assay.

B. Variation Between Parallel Determinations of Glucose Free Fatty Acids and Insulin in the plasma.

TABLE 25

OPTICAL DENSITY OF PLASMA GLUCOSE DUPLICATES IN INSULIN-SOMATOSTATIN AND INSULIN INFUSED POST-ABSORPTIVE DOG ($65 \leq t \leq 100$).

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between duplicates	1	$.45 \times 10^{-5}$	$.45 \times 10^{-5}$	.206
between samples	3	83.40×10^{-5}	27.80×10^{-5}	12.733
				$P < 0.01$
error	3	6.55×10^{-5}	2.18×10^{-5}	

TABLE 26

OPTICAL DENSITY OF PLASMA FREE FATTY ACIDS DUPLICATES IN INSULIN AND INSULIN-SOMATOSTATIN INFUSED POST- ABSORPTIVE DOG ($65 \leq t \leq 280$).

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between duplicates	1	1.78×10^{-3}	1.78×10^{-3}	2.21
between samples	8	66.92×10^{-2}	8.36×10^{-2}	103.83
error	8	6.44×10^{-3}	8.05×10^{-4}	$P < .005$

TABLE 27

ACTIVITY OF ^{125}I-INSULIN (COUNTS/4 MINUTES) DUPLICATES IN INSULIN-SOMATOSTATIN AND SOMATOTASTIN INFUSED POST ABSORPTIVE DOG ($195 \leq t \leq 270$).

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between duplicates	1	180266	180266.	.131
between samples	2	338033	169016	.123
error	2	2746321	1373160	