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ABSTRACT

The effectiveness of (insulin induced) hypoglycemic and (phlorizin induced) non-hypoglycemic glucoregulatory mechanisms were studied in conscious male rats by tracer methods using both non-compartmental and compartmental analysis. After the single intravascular injection of 3-H-3- and/or 14-C-U-glucose, a sum of two or three exponentials was fitted to the SA(t) curves of plasma glucose. Parameters were calculated from this curve.

Using the non-compartmental approach, the turnover rate (equal to the rates of appearance/disapppearance) increased by 56% over its basal value of 7.43 mg/kg.min when the level of plasma glucose was reduced to 40-45 mg/dl by the infusion of insulin. Thus, rats regulate effectively in response to hypoglycemia. Increased renal excretion of glucose due to phlorizin resulted only in a small and transient (30-35 minutes) increase in the glucose production to 8.04 mg/kg.min. Following this, the level of glucose in the plasma settled at 70-75 mg/dl. Accordingly, non-hypoglycemic glucoregulation does not operate efficiently in the rat. In diabetes, the turnover rate of glucose was found to be elevated to 8.69 mg/kg.min, 17% over baseline. No correlation between the size of the apparent volume of distribution and the level of plasma insulin was detected by non-compartmental analysis.

In normal rats, the arterial (aortic) injection of tracer, followed by arterial sampling (A/A) led to an estimated turnover rate 7% larger than when the injection of tracer into the artery was followed by taking samples from the vein i.e., the right heart (A/V). When tracer was injected into the vein and insulin was infused into the carotid artery together with sufficient glucose to maintain euglycemia, (i.e., a "glucose clamp" was applied), venous sampling (VAV) gave an accurate estimate of the rate of glucose infusion. When the AVA schedule was followed the calculated rate of turnover was 11% higher than the rate of glucose infusion. Accordingly, when equivalent tracer supply was observed (tracer and tracee entering the system at the same port), there was no significant difference between the turnover rates as calculated by the AAV and VVA procedures.

Compartmental analysis led us to evaluate various two and three compartmental models. The general two compartment model as proposed by Norwich (1977) was found to describe best the glucose system in the rat. The size of the first compartment was found to be about 165 ml/kg body weight and independent of plasma immunoreactive insulin (IRI) levels. The second compartment, however, increased in size from a basal 165 ml/kg to about 790 ml/kg in hyperinsulinemia whether or not hypoglycemia was prevented by the infusion of glucose ("glucose clamp"). Diabetes had no significant

effect on compartmental sizes. The robustness of the first compartment indicates that it may in fact be primarily a central distributing compartment the size of which is approximately equal to the extracellular volume and is not changed by plasma insulin. The second compartment on the other hand, may be considered to be an insulin sensitive compartment. Attempts to construct a three compartmental system, as found in man and dogs, have failed.

The insulin induced changes in apparent volume of the second compartment and in some of the rate constants, however, jeopardize the usefulness of the routine two compartment model with constant fractional exchange between compartments when Out of Dynamic Steady State (ODSS) experiments are analyzed. The use of a single compartmental representation of glucose distribution is advocated with a compartmental size set equal to V1 = 165 ml/kg. As an alternative a two compartmental representation with time dependent "rate constants" (Cobelli et al., 1987) may be employed to analyze the glucose system in rats.

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January 30, 1988

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CHAPTER I

INTRODUCTION:

I) REVIEW OF GLUCOREGULATION

I.A.) General Introduction

All tissues in the body utilize glucose as a source of energy at one time or another. Some tissues are obligate users, requiring a constant supply of glucose. These tissues include the erythrocytes, the renal medulla and the central nervous system (i.e., the brain). The brain can, however, use ketone bodies (acetoacetate and B-hydroxybutyrate) under conditions of extreme starvation. In the fed state, however, these compounds are present in very small amounts in the circulation (Rawn, 1983). Nevertheless, for the proper functioning of the CNS and inevitably the survival of the entire organism, the plasma glucose must be maintained at an adequate level in the system at all times. In mammals, the level of glucose in the plasma is maintained to within a very narrow range at about 90-120 mg/dl, or 5.0 to 6.7 mmole/dl (Guyton, 1986). Any minor deviations from this level will result in both neuronal and humoral mechanisms being activated, which will return the level to its original set point. This complex process by which the body maintains a constant plasma glucose concentration is known as GLUCOREGULATION.

I.B.) Insulin Induced Hypoglycemic Glucoregulation

Insulin is an anabolic hormone known for its potent hypoglycemic effects. Insulin causes an overall increased clearance of glucose from the plasma by: A) increasing the rate of glycogenesis (i.e., by activation of glycogen synthetase), B) enhancing the uptake of glucose into cells (i.e., by increasing carrier-mediated transport of glucose into cells, and by increasing the activity of the enzyme glucokinase which phosphorylates the glucose and prevents its exit once internalized), C) decreasing the rate of gluconeogenesis (i.e., by decreasing the quantities of liver enzymes required for gluconeogenesis), and D) activating the enzymes required for the conversion of excess glucose into fatty acids (Guyton, 1986). In man and dogs, the rise in endogenous glucose production seen in response to an i.v. injection or infusion of insulin, is an excellent example of a counterregulatory response to acute hypoglycemia (Rizza et al, 1979, Gauthier et al., 1980). Insulin induced hypoglycemia calls for an immediate increase of hepatic glucose production. The concurrent infusion of somatostatin, a peptide that inhibits the release of glucagon and insulin in man and dog has been shown to delay but not prevent the restitution of normoglycemia (Rizza et al., 1979; Gauthier and Hetenyi, 1982). On the other hand, if the release of both glucagon and catecholamines is inhibited during an insulin infusion, normoglycemia is prevented in man (Rizza et al., 1979) or dog (Gauthier and

Hetenyi, 1982). This indicates that in man and dogs it is the catecholamines, and not glucagon that play the major role in counterregulation to acute hypoglycemia. It should also be noted that cortisol and growth hormone (both of which are glucogenic) are also released in insulin induced hypoglycemia (Garber et al., 1976). Their importance in this counterregulation is, however, not critical because their action on gluconeogenesis unfolds slowly (Cryer, 1980). The glucoregulatory mechanisms in response to acute hypoglycemia in the rat have yet to be completely elucidated.

I.C.) Phlorizin Induced Non-Hypoglycemic Glucoregulation

Non-hypoglycemic glucoregulation, is the process of maintaining a constant plasma glucose level at normoglycemia in the face of an increased rate of removal of glucose from the circulation. The effect of non-hypoglycemic glucoregulation has been studied most effectively in mammals by the use of a polyhydroxyphenol glycoside known as phlorizin. Nash (1927) first showed that phlorizin could cause persistent glycosuria without hypoglycemia in mammals. Phlorizin has a high affinity for D-glucose receptors, believed to be part of the glucose transport system at the brush border of the proximal kidney tubule (Silverman and Black, 1975). When infused intravenously into the dog (6 ug/kg.min) phlorizin has been shown to completely block the reabosorption of D-glucose at the luminal surface of the

kidney (Silverman et al., 1970). At this rate of infusion, however, phlorizin has no effect on the hepatic D-glucose transport system in dogs. For this reason, phlorizin has been used in the past to increase the rate of removal of glucose from the circulation, in dogs in the post-absorptive (p.a.) state (Kolodny et al., 1962; Gauthier et al., 1980) or after a 4-day fast (Gauthier and Hetenyi, 1982), without interfering with the production of glucagon or the hepatic production of glucose (Goresky et al., 1974). It has been shown that an infusion of 50 ug/kg.min phlorizin into a dog causes the concentration of glucose in the plasma to be reduced by about 16% (Gauthier et al., 1980).

The rise in the endogenous glucose production rate due to a phlorizin infusion is mediated primarily by a release of glucagon (Gauthier et al., 1980, Gauthier and Hetenyi, 1982, Gauthier et al., 1983). It appears that in vivo, alpha cells are very sensitive to even a marginal decrease in the level of plasma glucose from normal. Consequently, the precise regulation of plasma glucose around the normal range is controlled by glucagon on glycogenolysis (Exton et al., 1969) and gluconeogenesis, especially from alanine (Chiasson et al., 1975) and glycerol (Hetenyi, 1981). It may be concluded from experiments in dogs that glucagon is the hormone primarily involved in non-hypoglycemic glucoregulation. The mechanisms for non-hypoglycemic glucoregulation in the rat have yet to be elucidated.

The rat has been used in a large number of experiments concerning glucose metabolism, yet few systematic studies on the distribution and kinetics of glucose, or on glucoregulation have been carried out to date. Cole et al. (1973) have shown that rats, unlike dogs, have an ineffective glucoregulation to overt insulin-induced hypoglycemia, and a relatively inefficient glucoregulation in response to an increased clearance of glucose by phlorizin. The rat's brain, not being as complex as the more advanced brain of dog or man, has been claimed to be less dependent on glucose, so the homeostatic responses may be different. In the newborn dog, for example, there is no increase in the rate of glucose production in hypoglycemia (Hetenyi et al., 1972). This seems to indicate that the brain of the newborn dog is less dependent on glucose than the brain of the adult dog. It may in fact be able to utilize other compounds such as short chain free fatty acids, ketone bodies or even other metabolites (Spitzer, 1975). Whether this holds true for the rat brain has not been determined.

The first aim of the experiments to be reported was to conduct a systematic study of the glucoregulatory mechanisms in the rat. Such research is possible, however, only if proper tracer techniques for the study of glucoregulation in the rat can be worked out. The best method for studying the glucoregulatory mechanisms in any organism is the use of

radiolabelled, or stable tracers (Norwich, 1977). Clearly, therefore, if some headway could be made concerning the use of tracers in the rat, it may become the preferred animal for such studies over man and dog, due to its relative inexpensiveness, ease of handling and care, availability, and facility for conducting ethically acceptable experiments. Therefore, the second aim of the experiments to be reported here was to establish the optimal kinetic model for glucose in the rat.

II) REVIEW OF TRACER METHODOLOGY:

II.A) General Introduction

Some metabolites are continuously being released into the circulation at a certain appearance rate (moles/min), and are simultaneously being removed therefrom at other sites at a certain disappearance rate (i.e., utilized or excreted - exhaled air, sweat, urine, etc.). These rates of appearance or disappearance may be constant or variable with time. This process is known as TURNOVER (Sheppard, 1962).

In general, the overall rate of release of a metabolite into the circulation is known as its rate of appearance (Ra). On the other hand, the rate of loss of a metabolite from the circulation is known as its rate of disappearance (Rd) (Wrenshall, 1955). It should be noted that only if excretion is excluded can Rd equal the rate of uptake by cells (Hetenyi et al., 1983b). If Ra=Rd, the metabolite is

said to be in a Dynamic Steady State (DSS) condition (Schoenheimer, 1943). Under basal conditions (e.g., postabsorptive state at rest) metabolites in the plasma are said to be in a DSS. Because, under such circumstances, the volume in which they are dissolved is also constant, DSS is reflected simply by a constant plasma concentration for the metabolite. Deviations from a set level of the metabolite in the plasma would indicate that the system is no longer in a DSS. Such a system is considered to be out of dynamic steady state (ODSS) (Hetenyi et al., 1983b). Such a condition usually evokes homeostatic mechanisms that result in the re-establishment of DSS.

Homeostasis is - somewhat superficially - equated with DSS. More precisely speaking, one has to realize that under the continuous influence of stimuli from the external world HOMEOSTASIS really includes a system that not only maintains but also re-establishes DSS in response to external disturbances. This is achieved by regulation of the rate of release and/or removal of the substance from the plasma (Cowan et al., 1973). This gives them a unique importance in the analysis of homeostasis. Homeostasis is therefore maintained by hormonal and nervous influences that affect the rates of release and removal of substances.

II.B) The Tracer Method:

As defined by Sheppard (1962), the tracer method is "a technique for observing a population of specific things such as molecules, living creatures or other entities by a

process of labeling". Observations on the labelled and nonlabelled elements as they mingle with one another yield information on the population as a whole (Hetenyi, 1986). Tracers allow one to obtain information about the rates of production and utilization, and about the distribution of a metabolite in an intact organism. The method is relatively non-invasive and is applicable to intact, unanesthetized animals or humans under ethically acceptable conditions.

The concentration of metabolites present in any body fluid is usually expressed as mmole or mg/ml. Since it is this metabolite's transport and turnover properties which we wish to trace, it is termed the tracee (Norwich, 1977). If one or more of the atoms of the tracee molecule are replaced with an isotope of these atoms, a molecule of tracer will result. Radioisotopes which emit beta particles and gamma rays are the most readily detectable, and are therefore the most frequently used in metabolic studies (Norwich, 1977). In this study, the term tracer means radioactive tracer. In the development of tracer theory, it is assumed that the tracer is "perfect" (i.e., it is, in every way, chemically and physically indistinguishable from the original metabolite to the biological system) (Norwich, 1977) .

The mass (M) and concentration (C) of tracee in biological studies are usually measured in units of mass or concentration, often as milligrams and mg/ml. Ideally, one would like to measure the mass (M*) and the concentration

(C*) of tracer in the same units. This, however, is not practical. The units of M* and C* are commonly measured in disintegrations per minute (dpm) and dpm/ml, respectively (Norwich, 1977).

II.C.) Calculations in Dynamic Steady State (DSS)

Following the injection or the infusion of tracer, samples of blood are taken at selected points of time. The concentration of tracer and tracee are determined in the plasma (i.e., C*, C). The ratio of C*/C in any given volume is referred to as the specific activity (SA) of the substance. The changes of any of these three variables with time are described by functions (most frequently exponentials) and are symbolized as C*(t), C(t) or SA(t) respectively. The statistical process of fitting an equation to an experimentally obtained set of data points is referred to as "curve fitting".

e.g., $C^*(t) = B1\ e^{-b1(t)} + B2\ e^{-b2(t)}$

(2 exponential equation for C*(t) observed in our experiments)

Using the tracer method, Ra refers to the rate at which tracee is released into the circulation from non-labelled sources. As mentioned, in DSS (i.e., a constant plasma level of tracee), Ra is equal to Rd, and is known as the turnover rate (Rt). Conceivably, once the tracer molecule is utilized (e.g., metabolized), the label which is released

can become reincorporated into the tracee and be released into the plasma. This phenomenon is called "recycling" of the label. If a label that does not recycle is selected, problems arising from recycling are avoided (Katz et al., 1974a).

The calculated turnover rate (Rt = Ra = Rd) of any tracee equals the rate at which nonlabelled molecules of the tracee enter the circulation. If the label is reincorporated into newly-released molecules of the tracee, the calculated Rt will underestimate the true rate of release of the tracee into the circulation and therefore will also underestimate the rate of utilization of the tracee in DSS (Katz et al., 1974a).

In studies on the glucose system, for example, the two most commonly used tracers are 3-H and 14-C. The use of 14-C labelled glucose for the calculation of turnover rate will result in an underestimation of the true Rt. This occurs because the 14-C label is able to recycle back to the glucose molecule via two interdependent cyles: A) the Cori Cycle (i.e., 14-C-glucose ----> 14-C-lactate -----> 14-C-glucose), and B) the glucose - alanine cycle (i.e., 14-C-glucose ----> 14-C-alanine ----> 14-C-glucose) (Katz et al., 1974b). Therefore, because of this recycling of the 14-C label, the calculation of the true turnover of glucose, which in DSS is equal to the rates of

appearance/disappearance (i.e., utilization of glucose -- metabolism and excretion), is underestimated. The apparent turnover rate as calculated by the 14-C-glucose is known as the irreversible disposal rate (Ri). The Ri is a measure of the rate at which glucose carbons leave the glucose pool (i.e., oxidation to CO_2, never to return (Hetenyi et al., 1983). In a DSS, Rt-Ri is also a measure of the rate of "de novo" synthesis of glucose: Ra from C-atoms that were not formerly part of circulating glucose. In our study, (Rt - Ri)/Ri has been taken as a measure of the contribution of gluconeogenesis to Rt (Norwich, 1977).

If a 3-H labelled glucose is used, this recycling problem may be avoided. This label is often released at some stage of metabolism of the tracer and is converted to 3-H_2O. Because the pool of water in the body is very large, the specific activity (SA) in plasma water remains low and therefore the recirculation of 3-H becomes negligible. Nevertheless, because of the operation of futile cycles, the later the step in the metabolism of the tracer at which 3-H becomes detached, the smaller the calculated apparent turnover rate (Katz et al., 1976). For example, 3-H-2-glucose as the tracer leads to a higher calculated Rt than 3-H-3- or 3-H-6-glucose would (Katz and Dunn, 1967; Katz et al., 1974a). This is because the 3-H from the second carbon atom in glucose is detached in a futile cycle operating between glucose-6-phosphate and fructose-6-phosphate, whereas 3-H from the third carbon atom is detached at the

triosephosphate isomerase step and the 3-H from the sixth carbon atom in the Krebs cycle (Dunn et al., 1976). The latter two tracers lead to approximately identical calculated turnover rates, because once the carbon atoms in glucose have passed the triose isomerase step they are either oxidized or returned to glucose only after passage through the Krebs cycle (Altszuler et al., 1975; Dunn et al., 1967).

In DSS, turnover rate can be determined either by tracer infusion or tracer injection methods. The two methods are necessarily equivalent (Hetenyi et al., 1983b). Using the tracer infusion method, Rt equals $R^*/\overline{SA}$ where R* (in dpm/min) equals the tracer infusion rate and $\overline{SA}$ refers to the steady time invariant specific activity in the plasma. Using the tracer injection method, the $Rt = MCR \times \overline{C}$. The metabolic clearance rate (MCR) is defined as $M^*/\int C^*(t)\,dt$ i.e., injected amount of tracer (dpm)/the area under the tracer concentration in the plasma decay curve with time from zero to infinity (DiStefano, 1976).

The validity of the calculated Rt using the infusion technique depends on the constancy of $\overline{SA}$ (i.e., the plasma concentration of C*/C is constant) throughout the system. Furthermore, it applies only if there is an equivalent tracer supply (i.e., tracer and tracee enter the system at the same point). The validity of the Rt calculation also depends on the rapidity of mixing of the tracer and tracee.

This approximation of the turnover rate may not be acceptable when substances (often with high relative turnover rates) enter the circulation at multiple points, whereas the tracer is infused at some other place at a single point. Sampling should to be from a point that can be taken to be at the "outflow" of the system: a vessel that collects the effluent blood from the metabolizing cells. Okajima et al. (1981) showed that the turnover of lactate was 47% greater when tracer was introduced into the artery and sampling was from the vein (A/V), when compared with a V/A design (infusion of the tracer into the vein and sampling from the artery). The A/V schedule puts the tracer and tracee into the same capillary bed and allows for the collection of mixed venous blood at the outlow. The same group showed that for glucose, however, a compound that enters the circulation at a single point into the vena cava, there was no significant difference between A/V and V/A designs. Therefore, the selection of the proper input and sampling sites may have a lesser or greater impact on the calculated turnover rate of a given metabolite.

The validity of the calculated Rt using the injection technique obviously depends on a constant plasma tracee concentration and on getting the correct value for the integral term for the C*(t) curve. It is imperative, therefore, that samples are taken both early in time (as soon as possible after the injection of tracer) and late in time (when 95-99% of the tracer has disappeared from the

plasma) in order to minimize errors arising from extrapolations between t = 0 and infinity. The tracer decay function obtained following injection can usually be adequately described by a sum to two or three exponentials or a power function (Norwich, 1977). The area under the curve may be estimated by planimetry, by weighing the paper representing the area, or analytically by using advanced iterative curve-fitting programs from which the area may be calculated by integration.

Several other terms are used to describe the dynamics of tracee in the body in DSS. The apparent volume of distribution equals the space in which tracer and tracee distribute themselves in the animal. The mean transit time (t) refers to the amount of time a tracer molecule (and therefore a tracee molecule) remains in the system (Norwich, 1977).

II.C.a.) Modelling

In tracer experiments, the total exchangeable mass (m) is determined as a measure of the total pool. The total exchangeable m is the amount of tracee in the body that rapidly intermixes with the tracer injected. Possibly, some molecules of the tracee never intermix with the tracer (nonexchangeable m), but for metabolites such as glucose, free fatty acids, plasma proteins, amino acids, and certain other compounds, nonexchangeable m is probably negligible (Hetenyi et al., 1983b). Even if all molecules of the

tracee intermix with the injected tracer they may do so at different speeds. Whereas the rapidity of this mixing covers a continuous spectrum ranging from very fast to very slow, the tracee nevertheless can be imagined as being distributed into discontinuous compartments, each of which has its own m, uniform SA and uniform Rt. By compartmental analysis, the apparent size, turnover characteristics, and connections of such compartments can be calculated. In this way, a model of the system (i.e., the whole body of the animal in question) may be built in which the system is represented as being composed of a varying number of compartments. Brown (1980) suggests that "a compartment is a vessel which contains a single distinct form of matter, and to which the law of conservation of matter applies. Compartmental models have often been of value in analysis of tracer experiments, especially those in which the rate of transfer of carbon atoms is followed quantitatively from one moiety to another (Shipley and Clark, 1972). In such an analysis the rates of exchange (flux) between compartments may represent different pathways for the transfer of carbon atoms from one metabolite to another. Compartments as determined by the behaviour of the tracer are operational, not morphological concepts; tracer-determined compartments of a model cannot be safely equated with any anatomical location (Norwich, 1977).

Upon injection of a radiolabelled tracer, if the SA(t) curve follows a monoexponential decay function, it can be

safely said that the tracer mixes, distributes itself, metabolizes and is lost from a single pool (Zilversmit, 1943). If however, the SA(t) curve follows a sum of two exponential decay functions for example, this would indicate that the tracer mixes, distributes, is metabolized and is lost from at least two distinct pools or compartments at two or more different rates. Similarly, a sum of three exponentials would indicate a three-compartmental, or an even more complex system (Brown, 1980).

According to Riggs (1963), to simplify the mathematical description of transfer between compartments, the following assumptions must be satisfied: a) The size, i.e., the volume, of each compartment remains constant, b) Each compartment is well stirred, so that any tracer or tracee entering the compartment is instantaneously distributed throughout the entire compartment, and c) The proportional rates of transfer between compartments in a multicompartmental model, remain constant.

II.D.) Calculations Out of Dynamic Steady State

For the study of the kinetics of a metabolite out of a steady state (i.e., plasma concentration of tracee varies with time) it is usual to use compartmental models. Norwich (1973) showed that one could calculate the rate of appearance (Ra) of glucose out of steady state using an infusion of tracer, where $Ra(t) = R^*(t)/\overline{SA}$, as long as as the SA of the metabolite in plasma remained constant. To

achieve this it however, was necessary to have prior knowledge of Ra(t).

Steele, in 1959 derived an equation to calculate the rates of appearance and disappearance of glucose from a one compartment system. Steele suggested that:

$$Ra(t) = (F - (p \times C(t) \times V \times dSA/dt)) \times 1/SA(t)$$

where, Ra(t) is the rate of appearance of glucose (mg/min), F is the rate of infusion of labelled glucose (dpm/min), p is the "pool fraction", V is the glucose distribution volume (ml), C(t) is the plasma glucose concentration (mg/dl) at time t, and SA(t) is the specific activity (dpm/mg) at time t. The rate of disappearance (Rd) was related to the Ra in the following way:

$$Rd(t) = Ra(t) - (p \times V \times dC(t)/dt)$$

where Rd is the rate of disappearance of glucose (mg/min).

Steele's method is based on the concept that all rapid departures from a steady state happen in a single rapidly-mixing compartment, which is a fraction of V (= pV). p is known as the pool fraction. The determination of the size of this pool fraction has been burdened with a great deal of difficulty. Steele (1959) initially suggested that for glucose about 50% of the entire intermixing mass is in the rapidly reacting fraction (p = 0.5) of the compartment, and exchange with the rest of the compartment was relatively slow. Cowan and Hetenyi (1971)

found p equal to 0.65 in dogs. Norwich et al. (1974) and Radziuk et al. (1974) found good agreement between the known rates of an unlabelled-inulin infusion and the rates calculated by a simultaneous labelled-inulin infusion when p was chosen to equal values between 0.5 and 0.8. Radziuk et al. (1978) repeated the same experiments, but instead of inulin used glucose at rates sufficient to reduce Ra to zero, in dogs, and adequate agreement between the rates infused and calculated by Steele's technique was obtained by taking p to equal 0.65 or 0.75. They found that the calculated Ra(t) differed from the actual infusion rates by an average of 9.5%. Recently, Proietto et al. (1987) conducted a set of euglycemic clamp experiments in rats and validated that a p value of 0.5 gave the most accurate estimate of the known rate of infusion of unlabelled glucose.

Steele's method of calculating the rates of appearance and disappearance out of steady state is based on the assumption that the tracee is distributed in a single compartment of size pV. Errors arising from this assumption can be minimized if the tracee is assumed to be distributed in two compartments. For human subjects and dogs, three-compartmental glucose systems have also been described (Foster et al., 1980; Cobelli and Toffolo, 1984; Ferrannini et al., 1985). Therefore, exchange of tracee and tracer between the rapidly and slowly reacting compartments can be taken into account in calculating the Ra and Rd into and out of the first (i.e. the rapidly reacting) compartment.

Norwich (1977) explains that two experiments must be performed. In the first experiment the rate constants governing the elimination of tracee from the first compartment and the exchange between the two compartments, as well as the V value of the first compartment, are determined by a preliminary tracer-injection experiment. In the second experiment the desired values of Ra and Rd are calculated under the experimental conditions to be investigated by using the rate constants obtained in the first experiment. This method has a great advantage over the Steele method in that no p value has to be assumed. A disadvantage of this technique however, is that two experiments must be carried out on the same subject, and one assumes that the rate constants remain the same during both experiments.

III.) THE CHEMICAL INDUCTION OF DIABETES

Alloxan (2,3,4,5 tetraoxohexahydropyrimidine) is a derivative of uric acid. The unique ability of alloxan to destroy the pancreatic beta-cells was first described by Dunn et al. (1943).

After a single diabetogenic dose injection of alloxan, a triphasic blood sugar response is observed. An initial elevation of blood sugar occurs 2-4h after the injection, followed by a period of marked hypoglycemia which may induce convulsions from 6-12h (Rerup, 1970), and finally a hyperglycemia at 24h (Fukuma et al., 1978; Cooperstein and Watkins, 1981).

The mechanism of the early hyperglycemia is not clear. This phase does not occur in fasted animals; however, alloxan will still induce diabetes in the fasted animal (Bell and Hye, 1983). Therefore, it is generally agreed that whatever alloxan does to the blood sugar during this early hyperglycemia phase is unimportant in relation to its diabetogenicity. It is generally agreed that the hypoglycemic response is related to the release of large amounts of insulin from the destroyed beta cells (Howell et al., 1967). The final stage is explained by the destruction of the beta cells, leading to an insulin deficiency (Lundquist and Rerup, 1967).

Although the evidence is overwhelming that alloxan causes diabetes by a direct toxic effect on the beta cells, the site of alloxan action and the exact mechanism of its toxicity are not completely understood. A number of studies have shown that alloxan acts primarily extracellularly by disrupting the integrity of the beta cell plasma membrane (Bell and Hye, 1983). The site at which alloxan interacts with the cell membrane is uncertain. Some evidence indicates that alloxan acts at the site for sugar transport into the beta cell. Therefore, alloxan may inhibit the D-glucose stimulated insulin release from the islets (Tomita et al., 1974). On the other hand, other evidence suggests that alloxan acts at a glucoreceptor site responsible for insulin release which is separate from the transport site

(Rossini et al., 1975). Still further evidence suggests that alloxan acts intracellularly by inhibiting a mitochondrial transport system for inorganic phosphate leading to a fall in intracellular pH and cell death (Boquist, 1980).

The chemical mechanism through which alloxan exerts its cytolytic effects is unknown. The generally held view is that in vivo the reduction of alloxan generates free radicals that could be cytotoxic to islet proteins, lipids and nucleic acids (Heikkila et al., 1974). Chemicals which can act as scavengers of free radicals, some metal chelators have been shown to protect against alloxan diabetes, and pretreatment of islets with the endogenous free-radical scavenger superoxide dismutase is likewise protective (Fischer and Hamburger, 1980).

One of the most intriguing features of alloxan is its high degree of selectivity for the beta cell. Recently, it has been suggested that this high degree of selectivity may be due in part to the great number of "ionized SH groups related to insulin release", which are especially sensitive to free radical attack, in the plasma membrane of the beta cell (Watkins et al., 1979).

Alloxan may be administered by a number of routes, but the intravenous one is the most effective (Bell and Hye, 1983). The optimal diabetogenic dose is 40-45 mg/kg (Bell and Hye, 1983).

IV) RESEARCH OBJECTIVES

The aims of this project were to perform a systematic study of the distribution and kinetics of glucose using tracers, and to evaluate the effectiveness of hypoglycemic and non-hypoglycemic glucoregulation in the rat. We were attempting to establish the mechanism of glucose kinetics in the rat, by employing non-compartmental and compartmental approaches in DSS and applying what we learned to the ODSS situation.

In experiments performed in "Dynamic Steady State" (DSS) we looked primarily at four problems:

1) The importance of the site of sampling and/or tracer administration on glucose kinetic parameters.
2) The effect of insulin on glucose kinetic parameters at a steady euglycemic or hypoglycemic level of glucose in the plasma.
3) The effectiveness of non-hypoglycemic glucoregulation in the rat. (i.e., the effect of phlorizin induced increased clearance of glucose on glucose kinetic parameters).
4) The effect of diabetes on glucose kinetic parameters.

In experiments performed "Out of Dynamic Steady State" (ODSS) we looked at two problems:

1) The effect of insulin infusion on the rate of appearance of glucose when the concentration of glucose in the plasma is falling.
2) The effect of phlorizin infusion on the rate of appearance of glucose in the early phase of the infusion.

CHAPTER II

MATERIALS AND METHODS

I) ANIMALS

Experiments were carried out on male Sprague-Dawley rats of 345 - 535 grams body weight for normal rats, and 302 - 410 grams body weight for diabetic rats. The animals had free access to tap water and food pellets (26% protein, 9% fat, 62% carbohydrate, 3% fibre, on a dry weight basis) and were housed at 20-21 °C with a 12-h light (07:00 to 19:00)/12-h dark cycle. Food was withdrawn one day prior to the experiment.

I.A) Chemical Induction of Diabetes

Diabetes was produced by the intravascular injection of 35 mg/kg alloxan (Issekutz, 1974) dissolved in 0.1 M acetate buffer, pH 4.4. The rats were used 72h after the injection of alloxan, and only if the postabsorptive plasma glucose level was above 250 mg/dl.

I.B) Protamine Zinc Insulin (PZI) Treatment

24-h prior to the experiment, seven rats were injected with 3 U of PZI (long-acting insulin), subcutaneously. Rats were then allowed free access to 10% glucose solution until the start of the experiment.

I.C) Cannulation - Surgery

Three days before the experiment, rats were cannulated

with one, two or three cannulae depending on the experimental scheme. All rats had a single cannula (Clay Adams PE-50) inserted 38-42 mm into the left carotid artery (Popovic and Popovic, 1960). The double and triple cannulated rats also had one PE-50 or two PE-20 (stretched Tygon tubing) cannulae respectively, inserted 38-42 mm into the right external jugular vein (Cunnigham et al., 1985). All cannulations were performed using a cut-down ligation technique, with the animal under a general anesthesia induced by an intra-peritoneal injection of sodium pentabarbitol (50 mg/kg). The cannulae were then tunnelled sub-cutaneously and exteriorized on the dorsal side of the neck just posterior to the ears through a small incision of about 3 mm. Prior to closure, the cannulae were flushed first with 20 U/ml heparin:saline (0.9%) and finally filled with a 500 U/ml heparin:saline solution. Note that diabetes was induced in some rats by injecting 35 mg/kg alloxan intra-arterially just prior to final flushing of the cannula with 500 U/ml heparin:saline. The ends of the cannulae were then either knotted or plugged with metal pins to prevent reflux of blood. The incisions were closed with 3-0 silk sutures.

II) PROCEDURES

Overnight-fasted rats were weighed and transferred into metabolic cages. The exteriorized cannulae at the neck were clamped and the knot and/or pins were removed. Pieces of

PE-50 cannulae (30-50 cm) filled with 20 U/ml heparin:saline and with a hollow metal tip were then inserted into the ends of the cannulae that were exteriorized from the neck of the rat. The cannulae were then flushed with 20 U/ml heparin:saline so that blood could be easily withdrawn from the rat.

II.A) Blood Samples

Blood samples 0.4 - 1.0 ml were drawn as needed from the vena cava or from the aorta using a 1 ml syringe (Becton-Dickinson) and then immediately transferred into plastic (1.5 ml) Eppendorf tubes containing dry heparin. The tubes were inverted a few times to mix the blood with the heparin to prevent clotting. The sampling cannula was flushed with an equivalent volume of 20 U/ml heparin:saline after each sampling. No more than 5 minutes elapsed between blood sampling and centrifugation in an Eppendorf centrifuge for 5 minutes. In most experiments, the red blood cells were resuspended in 0.9% saline and re-injected within 15 minutes.

II.B) Preparation of Blood Samples for Hormone and Metabolite Determinations

Blood samples of 0.5 ml were used for the determination of glucose concentration and specific activity (SA). The blood samples were centrifuged at 2,000 rpm (revolutions per minute), about 1000 x g, for 5 minutes in an Eppendorf microcentrifuge. The plasma was then removed and separated

into two aliquots of 25 ul and 200-300 ul, the former being used for tracee glucose determination and the latter for tracer glucose determination.

Blood samples of 0.5 ml were drawn for insulin determinations. 0.20 to 0.25 ml of plasma were frozen at - 20 °C and later transferred to -70 °C until the insulin assays were performed.

III) EXPERIMENTAL DESIGN

Two series of experiments were carried out. The first series dealt with experiments in Dynamic Steady State (DSS). The second series dealt with experiments Out of Dynamic Steady State (ODSS).

III.A BLOOD SAMPLING SCHEDULE

The experiments in DSS had two separate groups with respect to the blood sampling schedule. Tracer(s) was/were injected at t = 0. One group of rats had 8 blood samples taken at 2, 5, 13, 25, 40, 70, 110, and 150 minutes after the start of the experiment (i.e,. 8 sample schedule). Glucose and radioactive glucose were determined in all samples. The 13 and 110 minute samples were also assayed for immunoreactive insulin. A second group of rats had 12 blood samples taken at 1, 2, 3, 5, 8, 13, 25, 40, 70, 110, 140, and 170 minutes after the start of the experiment (i.e., 12 sample schedule). Glucose and radioactive glucose

were determined in all samples. No insulin determination was performed in the 12 sample experiments.

In the ODSS experiments, there were 9 samples taken at 70, 80, 90, 95, 100, 110, 130, 150 and 180 minutes after the start of the experiment (i.e., 9 sample schedule). Glucose and radioactive glucose were determined in all samples.

III.B EXPERIMENTS IN DYNAMIC STEADY STATE

III.B.a) Normal Rats

Sixteen single cannulated (i.e., one PE-50 cannula inserted into the carotid artery) and four double cannulated (i.e., one PE-50 cannula inserted into the carotid artery, and a second PE-50 cannula inserted into the jugular vein) normal rats with a steady plasma glucose concentration between 113 and 152 mg/dl at the start of the experiment were used. At t=0, 25 uCi 3-H-3-glucose and 10 uCi 14-C-U-glucose was injected into the carotid artery and blood sampling was begun.

1) 8 rats had the tracer injected into the carotid artery (A) and the blood was sampled from the carotid artery (A). In these rats the 8-sample schedule was followed.

2) 8 rats had the tracer injected into the carotid artery (A) and the blood was sampled from the carotid artery (A). In these rats the 12-sample schedule was followed.

Both groups (1) and (2) are referred to as A/A sampling experiments.

3) 4 rats had the tracer injected into the carotid artery (A) and the blood was sampled from the jugular vein (V). This was known as the A/V group. In these rats the 8 sample schedule was followed.

III.B.b) PZI-Treated Rats

Seven rats were cannulated (i.e., one PE-50 cannula inserted into the carotid artery) and injected with 3 U of a Protamine Zinc Solution as described on page 24. The rats were normoglycemic with a steady plasma glucose concentration between 100 and 152 mg/dl at the start of the experiment. At t=0 the rats were injected with 25 uCi 3-H-3-glucose and 10 uCi 14-C-U-glucose into the carotid artery and blood was sampled from the carotid artery. In these experiments the 8-sample schedule was followed.

III.B.c) Insulin Infused Rats

To nine double cannulated (i.e., one PE-50 cannula inserted into the carotid artery and another inserted into the jugular vein) rats, 50 mU/kg of a crystalline insulin solution was injected followed by a 12 to 15 mU/kg.min of a crystalline insulin solution (dissolved in 0.9% saline) infusion at a rate of 0.028 ml/min with either a Sage syringe pump (model #335) or a Harvard Instruments syringe pump (model #905) into the jugular vein until a steady

Figure 1: Methodology followed in Dynamic Steady State (DSS) Experiments

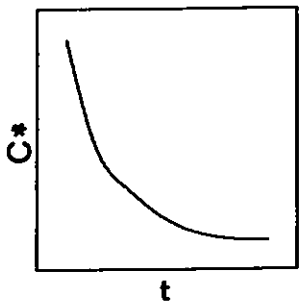
METHODS

3-H-3-glucose
and/or
14-C-U-glucose
Injected or Infused

↓

C*(t) fitted

↓



$$C^*(t) = A\, e^{-at} + B\, e^{-bt}$$

↓

Plasma Clearance Rate (PCR) = $M^*/\int C^*(t)dt$ (ml/min)
Turnover Rate (RT) = PCR(3-H) * $\bar{C}$ (mg/min)
Irreversible Disposal Rate (RI) = PCR(14-C) * $\bar{C}$ (mg/min)
Distribution Volume (VT) = M^*/B (ml)

Note: All values are expressed in per kilogram basis.

hypoglycemic glucose level was reached. The rats were hypoglycemic after 45 to 60 minutes reaching a steady plasma glucose concentration between 33 and 69 mg/dl. At this time (t=0) 25 uCi 3-H-3-glucose and 10 uCi 14-C-U-glucose were injected into the carotid artery and blood was sampled from the same carotid artery. In these rats, an 8-sample schedule was followed.

III.B.d) Euglycemic Glucose Clamp

To twenty triple cannulated (i.e., a single PE-50 cannula inserted into the carotid artery, and two PE-20 cannulae inserted into the jugular vein) rats, 50 mU/kg of a crystalline insulin solution was injected, followed by a 12 to 15 mU/kg.min of a crystalline insulin solution, and 32 to 36 mg/kg.min of a glucose solution infusion until a steady glucose level between 84 and 149 mg/dl was reached in the plasma. At this time, t=0, these rats were injected with 25 uCi 3-H-3-glucose and 10 uCi 14-C-U-glucose. The 8-sample schedule was followed.

1) 6 rats had the tracer glucose injected into the carotid artery (A), the tracee glucose (and insulin solution) infused into the jugular vein (V), and blood sampling was from the carotid artery (A). This was the AVA mode of sampling.

2) 4 rats had the tracer glucose injected into the jugular vein (V), the tracee glucose (and insulin

solution) infused into the carotid artery (A), and blood sampling was from the jugular vein (V). This was the VAV mode of sampling.

3) 6 rats had the tracer glucose injected into the carotid artery (A), the tracee glucose (and insulin solution) infused into the carotid artery (A), and blood sampling was from the jugular vein (V). This was the AAV mode of sampling.

4) 4 rats had the tracer glucose injected into the jugular vein (V), the tracee glucose (and insulin solution) infused into the jugular vein (V), and blood sampling was from the carotid artery (A). This was the VVA mode of sampling.

III.B.e) Phlorizin Infused Rats

Six double cannulated (i.e., a single PE-50 cannula inserted into the carotid artery, and another into the jugular vein) rats were infused with 50 ug/kg.min of phlorizin into the jugular vein, until a steady glucose level was reached. The rats were slightly hypoglycemic after 150 minutes, reaching a steady plasma glucose concentration between 69 and 81 mg/dl at the start of the experiment. At t=0 (about 150 minutes into the phlorizin infusion) these rats were injected with 25 uCi 3-H-3-glucose and 10 uCi 14-C-U-glucose into the carotid artery and blood was sampled from the carotid artery. Again, the 8-sample schedule was followed.

III.B.f) Diabetic Rats

Seven single-cannulated (i.e., a single PE-50 cannula was inserted into the carotid artery) rats in the diabetic state (produced by the intra-arterial injection of 35 mg/kg of alloxan at the time of cannulation), with a plasma glucose concentration between 287 and 922 mg/dl at the start of the experiment were used. At t=0 the rats were injected with 25 uCi 3-H-3-glucose and 10 uCi 14-C-U-glucose into the carotid artery and blood was sampled from the carotid artery. In these rats the 8-sample schedule was followed.

III.C.) EXPERIMENTS OUT OF DYNAMIC STEADY STATE

III.C.a) Insulin Infused Rats

Six triple-cannulated (i.e., a single PE-50 cannula inserted into the carotid artery and two PE-20 cannulae inserted into the jugular vein) rats with an initial plasma glucose between 100 and 140 mg/dl were used. At t=0 a priming injection of 10 uCi of 3-H-3-glucose was administered into the jugular vein. At the same time, an infusion of 3-H-3-glucose at a rate of about 850,000 dpm/min (0.38 uCi/min) and an infusion of 0.9% saline at 0.028 ml/min into the jugular vein was begun. At t=90, the saline infusion was switched to an infusion of 12-15 mU/kg.min (0.028 ml/min) of a crystalline insulin solution. Blood samples were taken from the carotid artery. In these rats the 9-sample schedule was followed.

III.C.b) Phlorizin Infused Rats

Six triple-cannulated (i.e., a single PE-50 cannula inserted into the carotid artery and two PE-20 cannulae inserted into the jugular vein) rats with an initial plasma glucose between 100 and 140 mg/dl were used. At t=0, a priming injection of 10 uCi 3-H-3-glucose was administered into the jugular vein. At the same time, an infusion of 3-H-3-glucose at a rate of about 850,000 dpm/min (0.38 uCi/min) and an infusion of 0.9% saline at 0.028 ml/min into the jugular was begun. At t=90, the saline infusion was switched to an infusion of 50 ug/kg.min (0.028 ml/min) phlorizin. Blood samples were taken from the carotid artery. Again, the 9-sample schedule was followed.

IV) THE DETERMINATION OF THE CONCENTRATION OF METABOLITES AND HORMONES IN THE PLASMA

IV.A.) The Determination of the concentration of Plasma Glucose (mg/dl)

The concentration of plasma glucose was determined from 10 ul samples using a Beckman Glucose Analyzer 2 (Beckman Instruments Inc., Fullerton, CA). The Analyzer utilizes an electrode, a reagent and an electronic system that measures the rate of change in oxygen consumption when a sample is injected into an enzyme reagent solution. When the solution is injected into the enzyme reagent solution, beta-D-glucose

from the sample combines with dissolved oxygen from the solution, according to the reaction:

$$\text{B-D-glucose} + O_2 \text{ ----------> gluconic acid} + H_2O_2$$

In this reaction, oxygen and glucose react in equimolar ratios to form gluconic acid and hydrogen peroxide. Therefore, because the system is saturated with oxygen, the number of moles of glucose in the injected sample is equal to the number of moles of oxygen utilized (Kadish et al., 1968). Because oxygen consumption rather than peroxide formation is measured, the reverse reaction leading back to oxygen must be prevented. The addition of ethanol, iodide and molybdate to the enzyme reagent cause the irreversible destruction of peroxide.

$$\underline{\text{i.e.,}}\quad H_2O_2 + \text{Ethanol} \xrightarrow{\text{catalase}} \text{Acetaldehyde} + H_2O$$

$$H_2O_2 + 2\,H^+ + 2\,I^- \xrightarrow{\text{molybdate}} I_2 + 2\,H_2O$$

IV.B.) The Determination of the concentration of 3-H-3-glucose and 14-C-U-glucose in the plasma (C*, dpm/ml)

The concentration of 3-H-3-glucose and 14-C-U-glucose in each sample was determined in terms of disintegrations per minute (dpm) of ionizing beta-radiation emitted by the

3-H and 14-C isotopes. (Note: In general, the early time points required only 0.2 ml of plasma as the C* was very high, whereas the later time points required up to 0.5 ml of plasma as the C* was lower).

The third carbon of glucose gives rise to the first carbon of lactate and other metabolites which can then recycle back into glucose via pyruvate (Cori Cycle) (Rawn, 1983). 14-C labeled non-glucose compounds (i.e., 14-C-lactate) are, therefore, removed from the plasma by passing through an anion exchange resin (BioRad AG 1-X8) (Reilly, 1975; Allsop et al., 1978). The tritiated water is removed from the system by deproteinizing the plasma with barium hydroxide ($Ba(OH)_2$) and zinc sulfate ($ZnSO_4$) according to Somogyi (1945). A 1 - 4 ml aliquot of dilute deproteinized plasma was evaporated <u>in vacuo</u> at 80°C until dryness (Hetenyi and Mak, 1970; Altszuler et al., 1975). The residue was reconstituted with 1 ml of distilled water, 10 ml of Scinti Verse (Fisher Scientific, Fairlawn, NJ) and counted in a Nuclear Chicago Mark II Scintillation Counter (Nuclear Chicago Corp., Des Plains, IL) set for double label counting for 3-H and 14-C (Hetenyi and Mak, 1970) for 40 minutes at 4°C. Correction for quenching and for quantification of the spillover of 14-C into the 3-H channel, respectively, were assessed by internal standardization with 14-C-toluene and 3-H-toluene.

IV.C.) The Determination of Immunoreactive Insulin (IRI) Concentration in the Plasma (uU/ml)

A kit for the determination of the plasma immunoreactive insulin based on the insulin radioimmunoassay (RIA) method as described by Herbert et al., (1968) was purchased from Pharmacia Diagnostics (Piscataway, NJ). Insulin determinations were carried out for the experiments in DSS.

This method is based on the competitive binding principle. Insulin in the sample competes with a fixed amount of 125-I labelled insulin for the binding sites of the specific antibodies. The reaction of insulin and antibody can be written as follows:

$$A + Ab \underset{K\text{-}1}{\overset{K1}{<\!\text{----------}\!>}} A\text{-}Ab$$

$$A^* + Ab \underset{K\text{-}2}{\overset{K2}{<\!\text{----------}\!>}} A^*\text{-}Ab$$

where A is the unlabelled antigen (insulin), A* is the 125-I labelled antigen (labelled insulin), A-Ab is the antigen-antibody complex, A*-Ab is the labelled antigen-antibody complex, K1, K2 are the association rate constants and K-1 and K-2 are the dissociation rate constants. It is assumed that K1 = K2 and that K-1 = K-2. Labelled and unlabelled insulin, therefore, compete for a fixed number of antibody sites. Thus, the greater the amount of unlabelled insulin in the sample to be assayed, the less the formation of the A*-Ab complex (Campfield et al., 1983). Following

separation of bound and free insulin, therefore, the radioactivity of the bound insulin can be determined in a gamma counter. The radioactivity is inversely proportional to the quantity of insulin in the sample. The relationship between the radioactivity of the sample and the actual insulin concentration in the sample was computed by means of a standard curve.

The insulin standards used in our assays were human insulin. The antibody used was raised in a guinea pig. The decanting suspension used to remove any free insulin following the Ag-Ab reaction was a Sepharose-anti-guinea pig IgG raised in sheep. The cross reactivity of the kit with bovine and porcine insulins was 100% by weight.

CHAPTER III

CALCULATIONS:

A double exponential function was fitted to the C*(t) decay function obtained from the timed plasma samples. Tracer was injected at t = 0. Time was measured as minutes. The BMDP-AR package program was used on an Amdahl 470 computer to arrive to an exponential decay function, by an iterative least squares procedure. This program also provided us with the standard deviations for the coefficients of the decay function.

$$\text{i.e. } C^*(t) = B1\, e^{-b1(t)} + B2\, e^{-b2(t)}$$

where: C* = dpm tracer glucose/ml plasma

I) DYNAMIC STEADY STATE CONDITIONS (DSS):

I.A.) Non-Compartmental Analysis:

1) Plasma Glucose: $\bar{C}$ = plasma glucose concentration (mg/ml)

2) Plasma Clearance Rate: $PCR = M^*/\int C^*(t)dt$ (ml/min)

where M* = injected amount (dpm) of tracer glucose

$\int C^*(t)dt$ = AREA below the C*(t) tracer decay curve

$AREA = B1/b1 + B2/b2$

3) Turnover Rate: $Rt = PCR(3\text{-}H) \times \bar{C}$ (mg/min)

where PCR(3-H) = the PCR for 3-H-3-glucose

4) Irreversible Disposal Rate: $Ri = PCR(14\text{-}C) \times \bar{C}$ (mg/min)

where PCR(14-C) = the PCR for 14-C-U-glucose

5) Total Volume of Distribution: (ml/kg)

$$Vt = M^* \times (\int tC^*(t)dt)/(\int C^*(t)dt)^2$$

where: $(\int tC^*(t)dt) = B1/(b1)^2 + B2/(b2)^2$

$(\int C^*(t)dt) = (B1/b1)^2 + (B2/b2)^2$

6) Mean Transit Time: MTT = Vt/PCR (min)

7) % Recirculation of Glucose (as % of Rt):

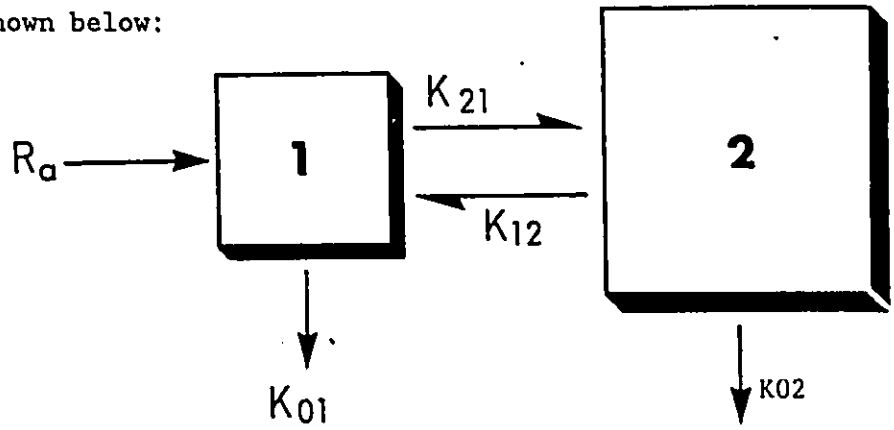
$$\%RECIRC = ((Rt-Ri)/Rt) * 100\%$$

Refer to Norwich (1977), DiStefano (1982), and Cobelli and Toffolo (1984) for the derivation of the formulae.

Note: All values have been normalized to a per kilogram basis.

I.B.) Compartmental Analysis:

The general configuration of the two compartment model is shown below:



1) Rate of flow of glucose from 1st to the 2nd compartment:

$$K21 = (B1 * (b1 - b2))/(B1 + B2) \quad (min^{-1})$$

2) Rate of flow of glucose from 2nd to the 1st compartment:

$$K12 = (B2 * (b1 - b2))/(B1 + B2) \quad (min^{-1})$$

3) Rate of irreversible loss of glucose from 1st compartment:

$K01 = b2 = K02$ by definition (min^{-1})

4) Volume of the first compartment: $V1 = M^*/(B1 + B2)$ (ml)

5) Total Volume of Distribution:

$$Vt = V1 + V2 = (1 + (K21/(K02 + K12))) \times V1 \text{ (ml)}$$

Assuming that: $K01 = K02$, and the concentration of glucose in the first compartment (C1) = C2.

6) Volume of the second compartment: $V2 = Vt - V1$ (ml)

7) 1st cpt. [glucose]: = plasma [glucose] = C1 (mg/ml)

8) 1st cpt. glucose mass: $M1 = V1 \times C1$ (mg)

9) 2nd cpt. glucose mass: $M2 = V2 \times C2$ (mg)

See Norwich (1977) and DiStefano (1982) for derivation of equations.

Note: All values except for the rate constants and the concentrations are nomalized to a per kilogram basis.

I.C.) K01 Clamp Calculations:

Here, K01 was assumed to equal that of the normal rats in all experimental groups. Therefore, K01 is a known constant. From this we derived the equations for K12, K21 and K02 as per Norwich (1977).

Let $Q = ((K01x(B1+B2)-((B2xb2)-(B1xb1)))/(b2-b1)$ = constant

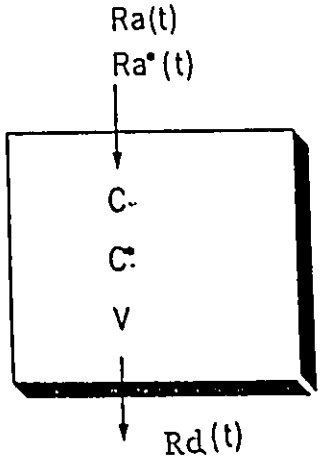
Therefore: $K02 = ((B2xb2)+(Qxb2)-(K01xb2))/Q$

$K21 = ((B1xb1)+(B2xb2)-(K01x(B1+B2)))/(B1+B2)$

$K12 = ((B1xb1)-((K21+K01)xB1))/Q$

II) CALCULATION OF Ra AND Rd OUT OF DYNAMIC STEADY STATE (ODSS)

II.A.) One Compartment Approximation:



1) Rate of appearance of glucose:

$Ra = (F - (p \times C(t) \times V \times dSA/dt)) \times 1/SA(t)$

where: F = infusion rate of 3-H-3-glucose (dpm/min)

p = pool fraction

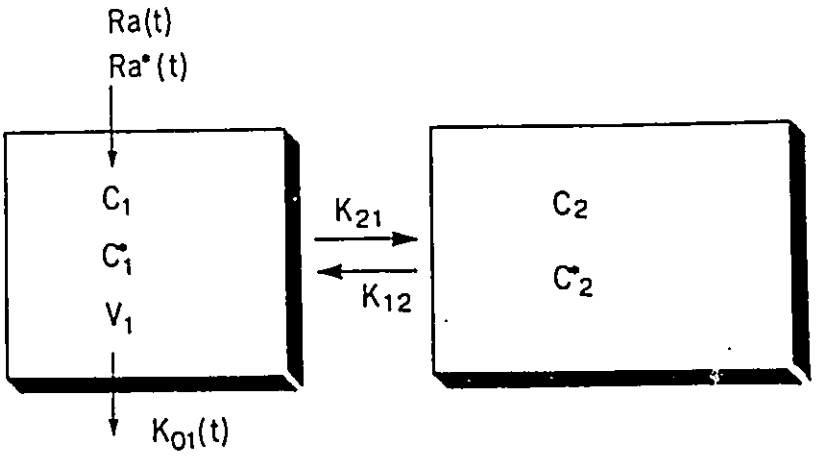
V = volume of distribution of glucose (ml/kg)

C(t) = plasma [glucose] at time t (mg/ml)

SA(t) = specific activity at time t (dpm/mg)

The Ra (mg/kg.min) was calculated using: (a) p = 0.5 (Proietto et al. 1987) and V = 294 ml/kg (the Vt obtained in normal rats using non-compartmental analysis), (b) p x V = 165 ml/kg (the size of the rapidly mixing compartment = V1 using compartmental analysis).

II.B.) Two Compartmental approximation:



where: C1* = concentration of labelled glucose in 1st cpt.

C2* = concentration of labelled glucose in 2nd cpt.

C1 = concentration of unlabelled glucose in 1st cpt.

C2 = concentration of unlabelled glucose in 2nd cpt.

V1 = volume of the 1st compartment

Ra(t) = rate of appearance of glucose at time t.

Ra*(t) = rate of appearance of tracer glucose at t.

K01(t) = rate constant for irreversible loss of glucose from cpt.1 at time t.

K12, K21 = rate constants for the cycling of glucose between cpt.1 and cpt.2.

Note: Kij are expressed as min^{-1}

1) $dC1^*/dt = Ra^*(t)/V1-(K21+K01(t))xC1^* + K12xC2^*$

2) $dC2^*/dt = K21xC1^* - K12xC2^*$

3) $dC1/dt = Ra(t)/V1-(K21+K01(t))xC1 + K12xC2$

4) $dC2/dt = K21xC1 - K12xC2$

In order to measure the non-steady state Ra(t) using the two compartment approximation, a preliminary experiment to evaluate K12 and K21 and V1 in DSS must be performed. Once these values have been calculated, they are assumed to remain constant during the experiment. A priming injection of the tracer (M*, dpm) is injected. This is followed by the infusion of the same tracer at a steady rate (R*, dpm/min) until a steady SA is reached in the plasma. This part of the experiment (usually of 2-h duration) is followed by the application of a disturbance. Once a perturbation takes the system out of DSS, concentrations of tracer glucose (C1*) and tracee glucose (C1) in plasma are measured at specific time intervals. Equations (1) through (4) can then be used to calculate the "unknown" rate of appearance of glucose Ra(t), at any time t.

Refer to Norwich (1977), and Steele et al. (1974) for the derivation of the formulae.

III) Statistical Analysis

. Means and standard errors of the mean (SEM) were calculated for each measured and calculated quantity for all animals. The Scheffe test (F) was utilized to perform a two-way analysis of variance, making all possible pairwise comparisons among means of samples of unequal sizes. The Dunnett test was used to compare one control sample to all the others. Paired t-tests were performed to determine significant differences of the relative of the measured and calculated parameters compared to control.

Paired and unpaired t-tests, as well as one-way and two-way analyses of variance were performed with the help of package programs (SPSSX and Minitab) run on the Amdahl mainframe computer at the University of Ottawa, and the program (TANOVA) run on the VAX computer at Carleton University (Snedecor, 1956; Colton, 1974; Roscoe, 1975).

IV) Chemicals

The 3-H-3- and 14-C-U-glucoses were purchased from New England Nuclear Canada (Lachine, PQ). Alloxan monohydrate and phlorizin were obtained from the Sigma Chemical Company (St.Louis, MO). Injectable crystalline and protamine zinc beef and pork insulins were supplied by Connaught Laboratories Limited (Willowdale, ON). The alpha-D-dextrose used to make the unlabelled glucose solutions was obtained from Fisher Scientific (Fairlawn, NJ). The Insulin RIA kit was obtained from Pharmacia Diagnostics (Canada) Inc. (Dorval, PQ). All of the chemicals used were obtained from Fisher Scientific (Fairlawn, NJ) and were of the highest grade and purity available.

CHAPTER IV

RESULTS:

I) EXPERIMENTS IN DYNAMIC STEADY STATE

I.A) Non-Compartmental Analysis

I.A.1) The Kinetics and Distribution of Glucose

Rats were observed in six different DSS conditions: namely the normal rats fasted overnight, rats treated with PZI, rats during the infusion of insulin in hypoglycemia or with plama glucose clamped near the euglycemic level, rats infused with phlorizin. All rats were injected with both 3-H-3- and 14-C-U- labelled glucose once a DSS state was reached as indicated by a constant plasma glucose. A sum of two exponentials function was fitted to the tracer decay function (Note: to some of the decay curves, a sum of three exponentials function was fitted (See Appendix I).

The plasma concentration of glucose and immunoreactive insulin (IRI) was determined in all rats. From the tracer decay function C*(t), the plasma clearance rate, the turnover rate of glucose, the irreversible disposal rate of glucose, the mean transit time of glucose and the overall total distribution volume of glucose were computed as described on page 40.

I.A.1.i) Plasma Glucose (Refer to Figure 2)

The plasma glucose concentration was not significantly

FIGURE 2: Plasma glucose (C) in Normal, PZI-treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infusion and 32-36 mg/kg.min glucose infusion), Phlorizin Infused (50 ug/ kg.min), and Diabetic rats. Ordinate is in mg/ml. Standard errors of mean are shown as vertical bars. (Exception: Diabetic Rats - Plasma glucose = 6.1 +/- 0.9 mg/ml).

Legend:

NORMAL = Normal Rats

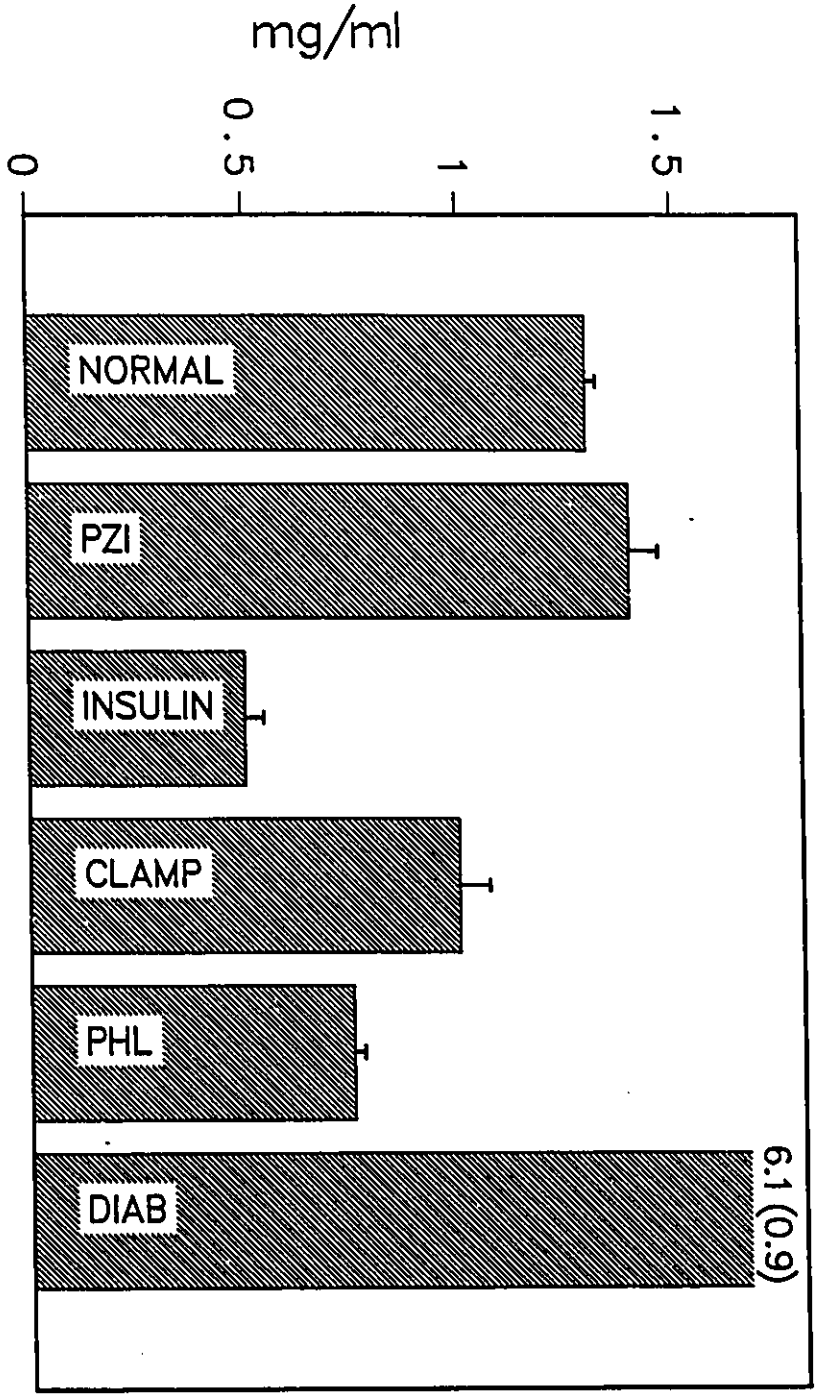
PZI = PZI-Treated Rats

INSULIN = Hypoglycemic Insulin Infused Rats

CLAMP = Euglycemic Insulin Infused Rats

PHL = Phlorizin Infused Rats

DIAB = Diabetic Rats



PLASMA GLUCOSE

different in normal rats (132 +/- 3 mg/dl) in comparison with either PZI-treated rats (140 +/- 7 mg/dl) ($F = 0.208$, n.s.) or euglycemic insulin infused rats (118 +/- 13 mg/dl) ($F = 2.088$, n.s.). The rats infused with insulin in which plasma glucose had been clamped glucose reached a DSS with respect to plasma glucose fairly quickly (after about 45-60 minutes of a 12-15 mU/kg.min insulin and 32-36 mg/kg.min glucose infusion) as did the rats infused with insulin alone. The rats infused with insulin alone were hypoglycemic (48 +/- 4 mg/dl) and differed significantly from normal ($F = 43.56$, $p < 0.001$), PZI ($F = 50.99$, $p < 0.001$) and clamped rats ($F = 34.267$, $p < 0.001$). The rats infused with phlorizin reached a DSS with respect to plasma glucose very slowly (only after about 150 minutes into a 50 ug/kg.min infusion). The plasma glucose in the PHL-infused rats (76 +/- 2 mg/dl) was significantly lower than the normal ($F = 12.36$, $p < 0.005$), PZI ($F = 18.66$, $p < 0.01$) or clamped ($F = 9.88$, $p < 0.005$) rats and significantly higher than the insulin infused rats ($F = 22.47$, $p < 0.001$). The alloxan-induced diabetic rats had a grossly elevated plasma glucose level (613 +/- 93 mg/dl) which differed significantly from all other groups in DSS ($F = 150.3$, $p< 0.001$).

I.A.1.ii) Immunoreactive Insulin (Refer to Figure 3)

The immunoreactive insulin (IRI) is a measure of the circulating level of insulin in the plasma. The plasma IRI in normal rats was 12.6 +/- .8 uU/ml. This was significantly lower ($F = 36.85$, $p < 0.01$) than the IRI found in the PZI-treated rats (24 +/- 4 uU/ml).

FIGURE 3: Plasma Immunoreactive Insulin (IRI) in Normal, PZI-Treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infusion and 32-36 mg/kg.min glucose infusion), Phlorizin Infused (50 ug/kg.min), and Diabetic Rats. Ordinate is in uU/ml. Standard errors of mean are shown as vertical bars. (Exceptions: Hypoglycemic Insulin Infused Rats - IRI = 630 +/- 216 uU/ml; Euglycemic Insulin Infused Rats - IRI = 531 +/- 89 uU/ml; Diabetic Rats - IRI = 0).

Legend:

NORMAL = Normal Rats

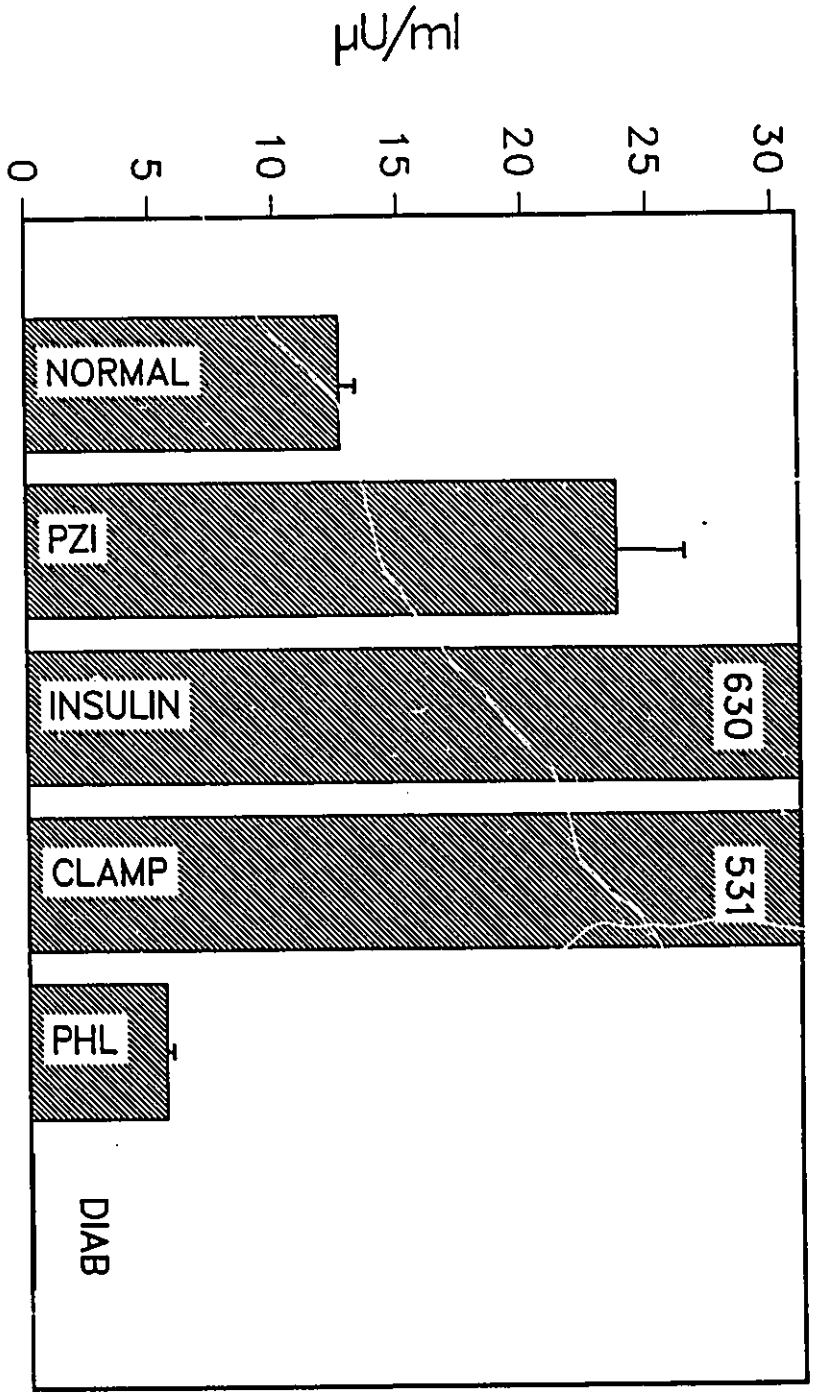
PZI = PZI-Treated Rats

INSULIN = Hypoglycemic Insulin Infused Rats

CLAMP = Euglycemic Insulin Infused Rats

PHL = Phlorizin Infused Rats

DIAB = Diabetic Rats



PLASMA INSULIN

The phlorizin infused rats had a plasma IRI concentration that had decayed to 5.5 +/- .2 uU/ml. This was significantly lower than in the normal ($F = 9.90$, $p < 0.05$) and PZI treated groups ($F = 49.46$, $p < 0.01$). The rats to which insulin was infused without or with a glucose clamp had the highest insulin titers of all the groups : 630 +/- 216 and 531 +/- 89 uU/ml. Their respective plasma IRI was significantly higher than in normals ($F = 299.1$, $p < 0.005$; $F = 233.5$, $p < 0.005$), PZI ($F = 263.9$, $p < 0.005$; $F = 205.9$, $p < 0.005$) or PHL rats ($F = 399.0$, $p < 0.001$; $F = 287.9$, $p < 0.005$). No insulin was detected in the plasma of diabetic rats. We therefore assumed their plasma IRI was near zero.

I.A.1.iii) Total Distribution Volume (Refer to Figure 4)

The total distribution volume (Vt) is a measure of the total space which glucose would occupy in the animal at a concentration equal to that in blood plasma. In normal rats the Vt was found to be equal to 294 +/- 20 ml/kg. This is equivalent to 29% of the total volume of the rat. Since the specific gravity of the animal is near unity; the volume was equated to mass and weight. In PZI rats, the calculated Vt was equal to 269 +/- 19 ml/kg, and in diabetic rats 243 +/- 44 ml/kg. In phlorizin infused rats the Vt = 287 +/- 34 ml/kg. In the insulin infused rats, Vt = 337 +/- 31 ml/kg in the presence of acute hyperinsulinemia and hypoglycemia. When plasma glucose was clamped, Vt = 301 +/- 40 ml/kg. It is very clear from these results that, as calculated by non-

compartmental analysis, the total volume of distribution of glucose does not change significantly under conditions of mild or gross hyperinsulinemia in the presence or absence of hypoglycemia, or under conditions of diabetes (hyperglycemia and the virtual absence of insulin) ($F = 2.022$, n.s.).

FIGURE 4: Total Volume of Distribution of Glucose (Vt) in Normal, PZI-Treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infused and 32-36 mg/kg.min glucose infused), Phlorizin infused (50 ug/kg.min), and Diabetic Rats. Ordinate is in ml/kg. Standard errors of mean are shown as vertical bars.

Legend:

NORMAL = Normal Rats

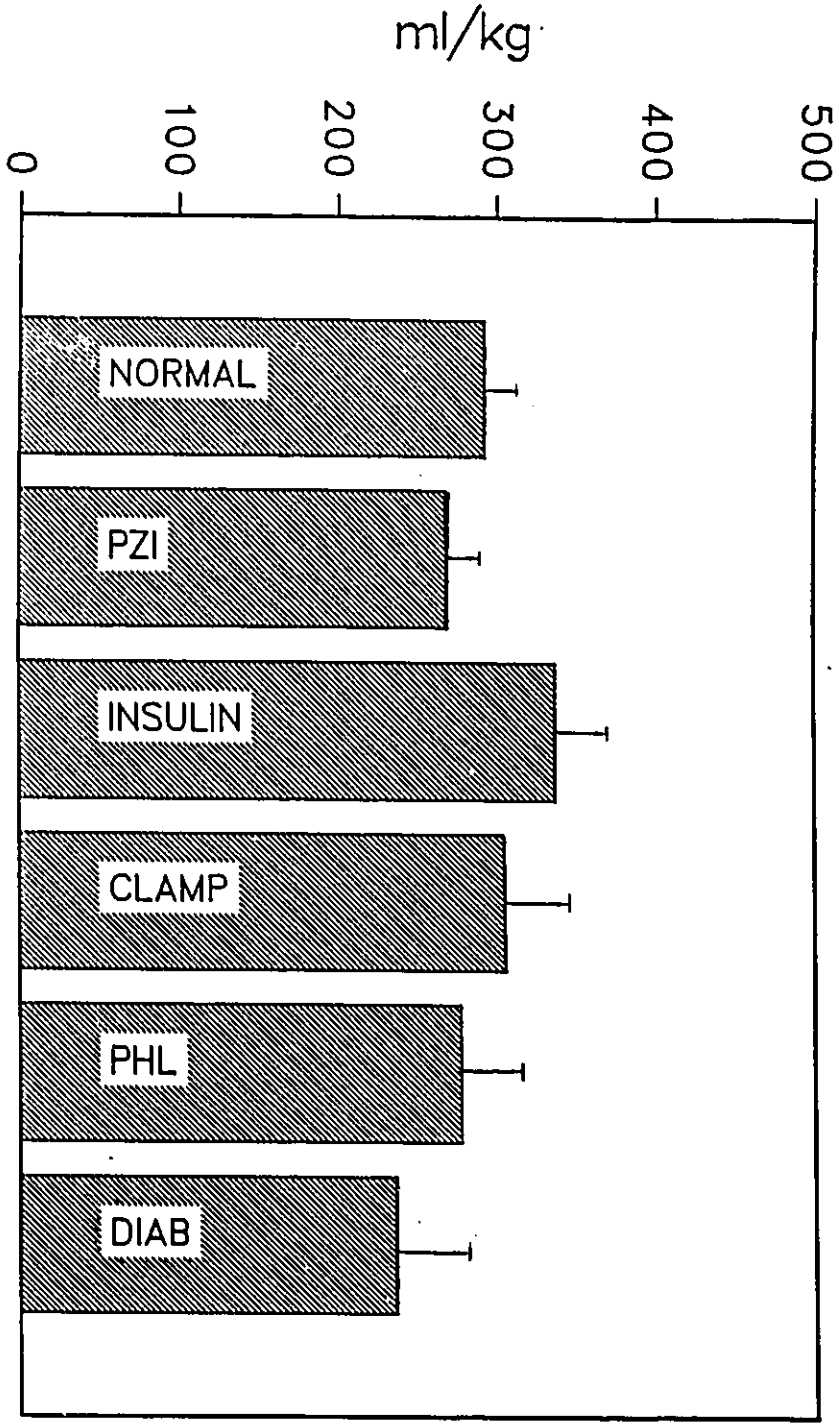
PZI = PZI-Treated Rats

INSULIN = Hypoglycemic Insulin Infused Rats

CLAMP = Euglycemic Insulin Infused Rats

PHL = Phlorizin Infused Rats

DIAB = Diabetic Rats



TOTAL VOLUME (Vt)

FIGURE 5: Plasma Clearance Rate (PCR) in Normal, PZI-Treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infused and 32-36 mg/kg.min glucose infused), Phlorizin Infused (50 ug/kg.min), and Diabetic Rats. Ordinate is in ml/kg.min. Standard errors of mean are shown as vertical bars. (Exceptions: Hypoglycemic Insulin Infused Rats - PCR = 26.7 +/- 3.1 ml/kg.min; Euglycemic Insulin Infused Rats - PCR = 35.2 +/- 4.8 ml/kg.min).

Legend:

NORMAL = Normal Rats

PZI = PZI-Treated Rats

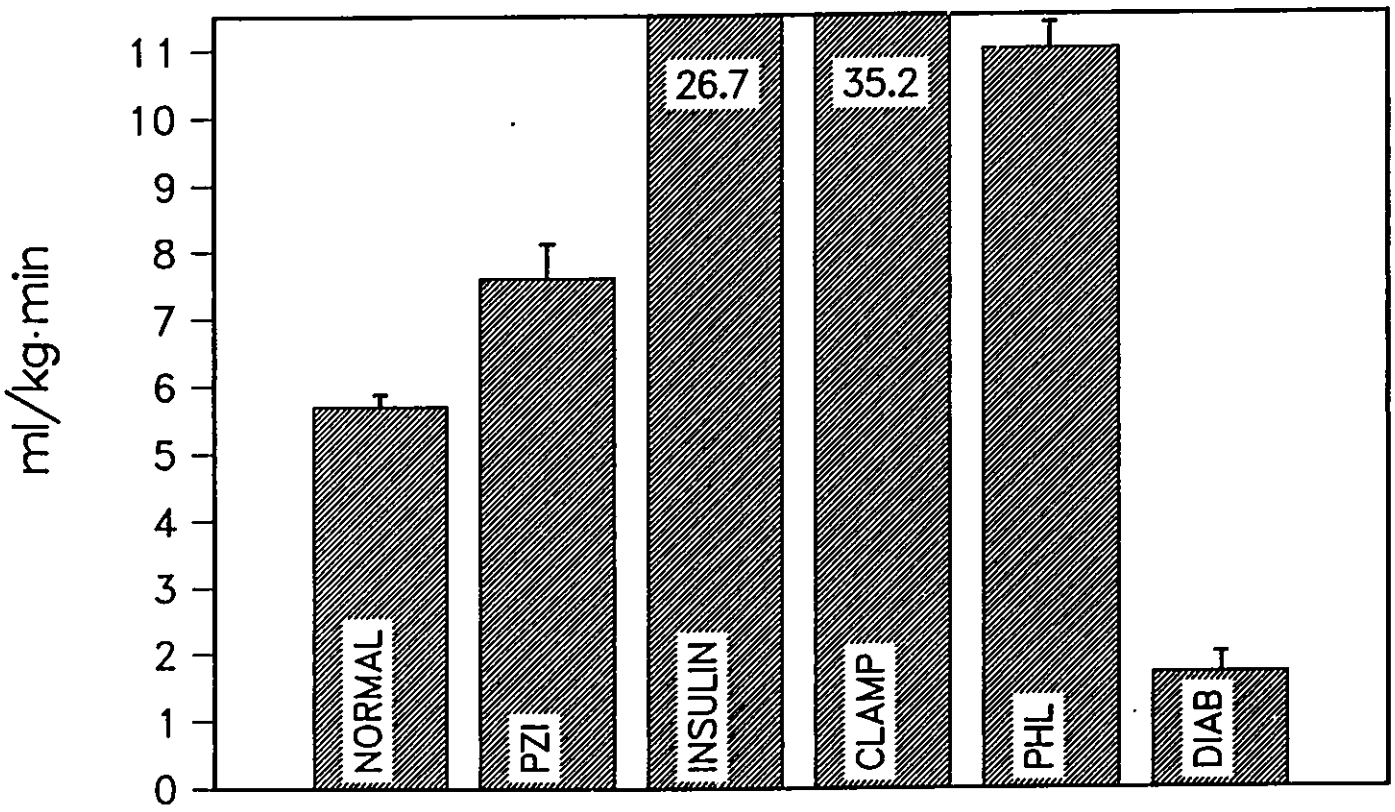
INSULIN = Hypoglycemic Insulin Infused Rats

CLAMP = Euglycemic Insulin Infused Rats

PHL = Phlorizin Infused Rats

DIAB = Diabetic Rats

PLASMA CLEARANCE RATE



I.A.iv.) Plasma Clearance Rate (Refer to Figure 5)

The plasma clearance rate (PCR) of glucose is the virtual volume of plasma cleared of glucose in unit time, usually one minute. The PCR is determined from the 3-H-3-glucose decay curve from the plasma as 3-H atoms do not recycle into plasma glucose. In normal rats, the PCR was calculated as 5.73 +/- 0.12 ml/kg.min. This was significantly lower than the PCR of PZI-treated rats (7.63 +/- 0.52 ml/kg.min) ($F = 17.53$, $p < 0.01$). The PCR was the highest in hyperinsulinemic at euglycemic glucose level ("clamped") (35.2 +/- 4.8 ml/kg.min) ($F = 11.09$, $p < 0.05$). This was significantly higher than the PCR in the insulin infused group (26.7 +/- 3.1 ml/kg.min) ($F = 23.37$, $p < 0.01$), and also higher than the PCR's of all the other groups ($F = 35.59$, $p < 0.01$). It should be noted that, despite the significant difference between the PCRs of the insulin infused hypoglycemic and euglycemic groups, they are 466% and 614% of the PCR in normal rats. Both of these groups, therefore, have grossly elevated clearance rates. The PCR in the phlorizin treated rats (10.67 +/- 0.79 ml/kg.min) was significantly greater than normal ($F = 48.97$, $p < 0.005$). The diabetic rats had the lowest PCR of all the experimental groups (1.66 +/- 0.29 ml/ kg.min), and was significantly lower than all other groups ($F = 20.66$, $p < 0.05$). The correlation between the PCR and the Plasma IRI was very high ($r=0.98$).

FIGURE 6: Turnover Rate of Glucose (Rt) in Normal, PZI-Treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infused and 32-36 mg/kg.min glucose infused), Phlorizin Infused (50 ug/kg. min), and Diabetic Rats. Ordinate is in mg/kg.min. Standard errors of mean are shown as vertical bars.

Legend:

NORMAL - Normal Rats

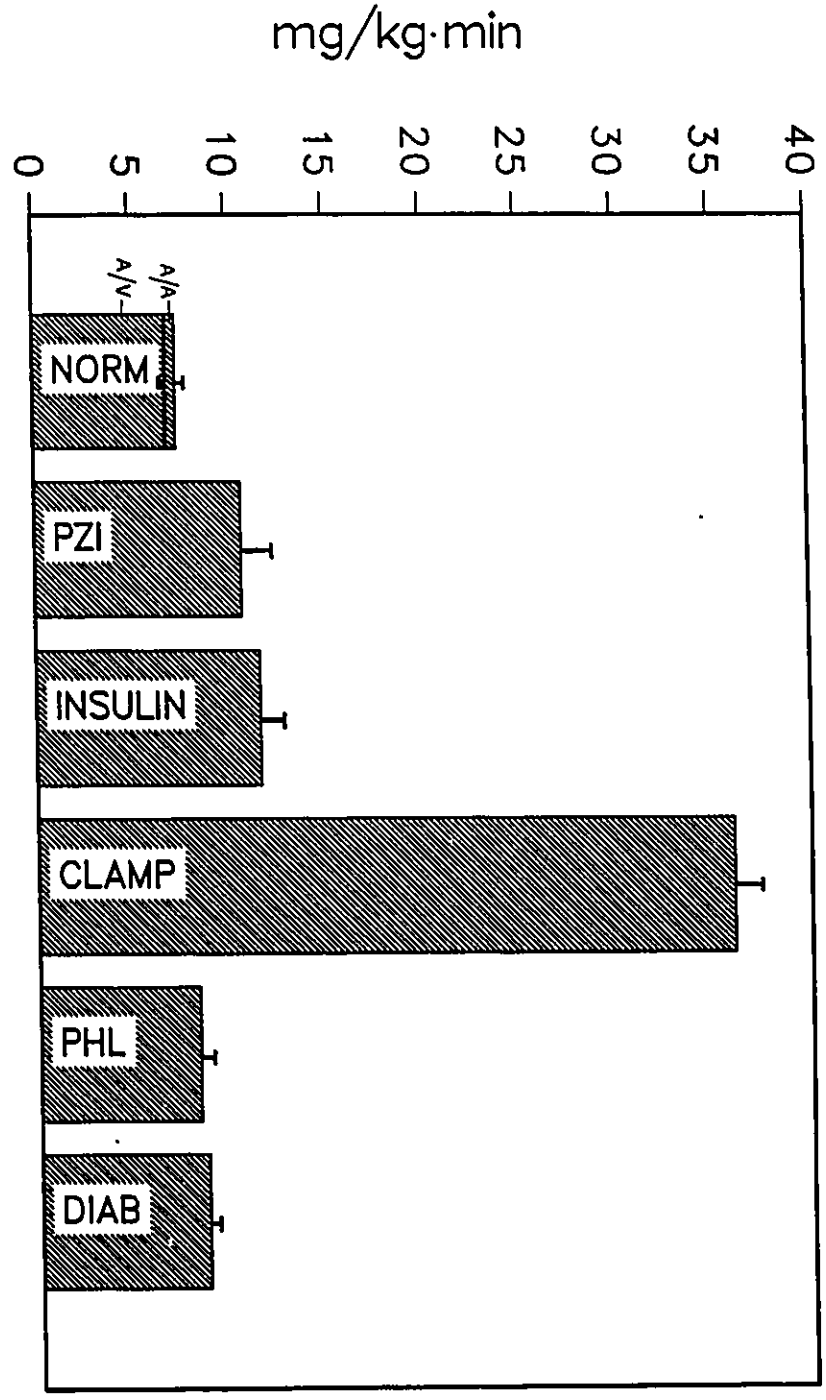
PZI - PZI-Treated Rats

INSULIN - Hypoglycemic Insulin Infused Rats

CLAMP - Euglycemic Insulin Infused Rats

PHL - Phlorizin Infused Rats

DIAB - Diabetic Rats



TURNOVER RATE (R_T)

I.A.1.iv.) Turnover Rate of Glucose (Refer to Figure 6)

The turnover rate (Rt) is a measure of the rate of appearance or disappearance of glucose into or out of the system in a DSS. As discussed on page 59, it is best calculated from the decay curve of the 3-H-3-glucose from the plasma. The turnover rate of glucose in normal rats is 7.43 +/- 0.15 mg/kg.min. The Rt in the PZI-treated group (10.69 +/- 0.96 mg/kg.min) was significantly higher (44%) than that of the normal group ($F = 34.09$, $p < 0.005$). The Rt in the insulin infused group (11.58 +/- 1.07 mg/ kg.min) was also significantly higher (56%) than the normal ($F = 23.79$, $p < 0.001$) but not than the Rt for the PZI treated group ($F = 1.397$, n.s.). It is interesting to note, therefore, that despite a gross elevation in the plasma IRI in the insulin infused group compred to the PZI group, there was no relative increase in the glucose production rate by the liver (Rt). The Rt for the clamped rats was significantly higher than that of all of the other groups, and for the most part equalled the infusion rate of glucose (36 mg/kg.min) ($F = 24.37$, $p < 0.01$). Please refer to the "I.A.2.ii) Methodological Studies" section of the Results for further details. The phlorizin infused group had an Rt (8.04 +/- 0.49 mg/kg.min) that was not significantly different from that of the normal rats ($F = 0.826$, n.s.). This inability of the rat to increase its glucose production rate in response to a phlorizin infusion was probably the cause of the moderate hypoglycemia seen in this group. The

Rt of the diabetic rats (8.69 +/- 0.39 mg/kg.min) was, as expected, significantly higher (17%) than that of the normal rats ($F = 6.87$, $p < 0.01$).

FIGURE 7: Irreverible Disposal Rate (Ri) of glucose in Normal, PZI-Treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infused and 32-36 mg/kg.min glucose infused), Phlorizin Infused (50 ug/kg.min), and Diabetic Rats. Ordinate is in mg/kg.min. Standard errors of mean are shown as vertical bars.

Legend:

NRM - Normal Rats

PZI - PZI-Treated Rats

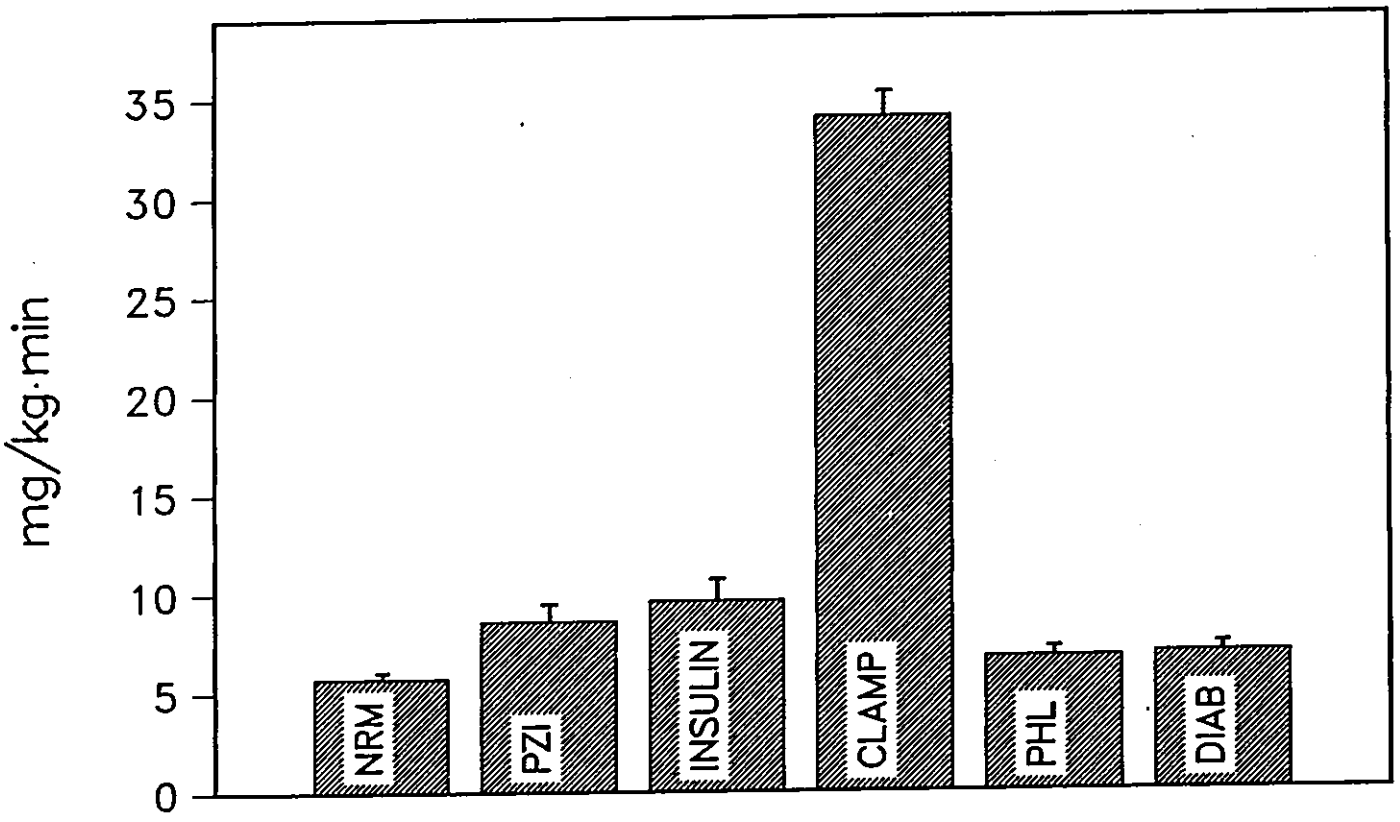
INSULIN - Hypoglycemic Insulin Infused Rats

CLAMP - Euglycemic Insulin Infused Rats

PHL - Phlorizin Infused Rats

DIAB - Diabetic Rats

IRREVERSIBLE DISPOSAL RATE (R_I)



I.A.1.vi) Irreversible Disposal Rate (Refer to Figure 7)

The irreversible disposal rate (Ri) is a measure of the rate of irreversible loss of glucose carbons from the system. It is calculated from the decay curve of 14-C-U-glucose. The Ri in normal rats was found to be 5.68 +/- 0.12 mg/kg.min. The Ri in PZI rats (8.62 mg/kg.min +/- 0.87) is significantly higher than in normal ($F = 34.67$, $p < 0.05$). In insulin infused hypoglycemic rats the Ri (9.60 +/- 0.97 mg/kg.min) is also significantly higher than normal ($F = 40.91$, $p < 0.01$) but not significantly higher than the PZI rats ($F = 0.807$, n.s.). Obviously, the highest Ri was seen in the hyperinsulinemic euglycemic group to which 32-36 mg/kg.min glucose was infused to maintain euglycemia. The Ri in phlorizin infused rats (6.71 +/- 0.37 mg/kg.min) was significantly higher than in normal rats ($F = 16.36$, $p < 0.01$). In diabetic rats the Ri (6.93 mg/kg.min +/- 0.39) was also significantly higher than normal ($F = 23.71$, $p < 0.05$) but not significantly different from that observed in the phlorizin treated group ($F = 1.100$, n.s.).

I.A.1.vii) Percent Recirculation of Glucose Carbons (Refer to Figure 8)

The percentage of recirculation of glucose carbons (%RECIRC) is a measure of the activity of the Cori and the glucose-alanine cycles. Both of these cycles are capable of returning carbon atoms originating from plasma glucose, back into newly synthesized glucose. The highest %RECIRC was seen in normal rats (23.5 +/- 1 %).

FIGURE 8: The Percent of Recirculation of Glucose Carbons back into newly synthesized glucose (%RECIRC) in Normal, PZI-Treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infused and 32-36 mg/kg.min glucose infused), Phlorizin Infused (50 ug/kg.min), and Diabetic Rats. Ordinate is in Percent (%). Standard errors of mean are shown as vertical bars.

Legend:

NORMAL = Normal Rats

PZI = PZI-Treated Rats

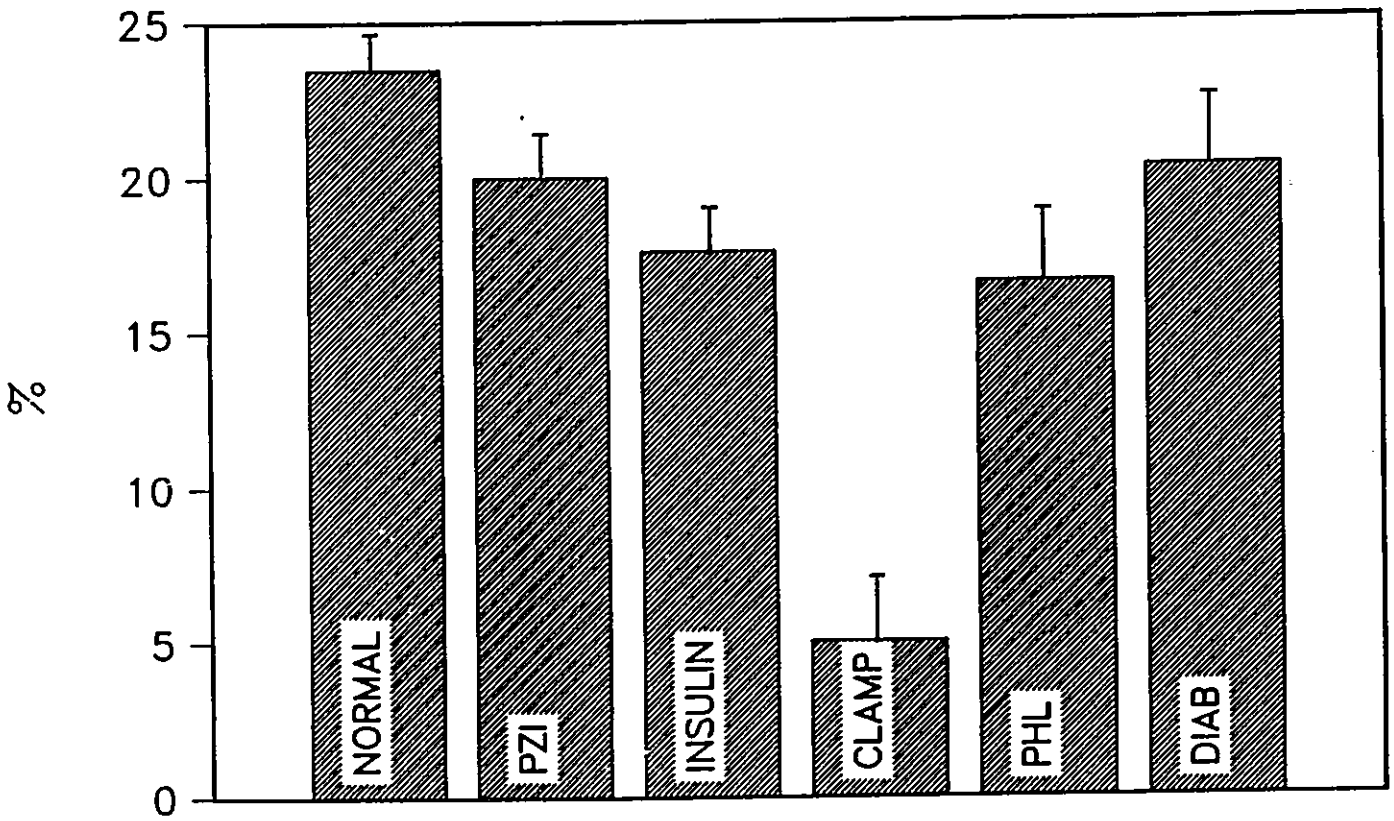
INSULIN = Hypoglycemic Insulin Infused Rats

CLAMP = Euglycemic Insulin Infused Rats

PHL = Phlorizin Infused Rats

DIAB = Diabetic Rats

PERCENT RECIRCULATION



The %RECIRC in PZI rats (20 +/- 1.4 %) was significantly lower than normal ($F = 16.69$, $p < 0.05$), as was the %RECIRC in insulin infused rats (17.6 +/- 1.3) ($F = 12.71$, $p < 0.01$). The PZI-treated and the insulin infused groups did not differ significantly from each other ($F = 1.808$, n.s.). The %RECIRC in the clamped rats (5 +/- 2 %) was the lowest of all of the groups , as was expected ($F = 89.75$, $p < 0.005$). This value was barely above the expected zero and may indicate a less than complete shut-down of hepatic glucose production. In phlorizin infused rats, the %RECIRC was 16.6 +/- 2.3 %. This was significantly lower than that of the normal rats ($F = 20.69$, $p < 0.01$). In diabetic rats, the %RECIRC was 20.3 +/- 2.3 %. This was significantly higher than the clamp rats ($F = 60.71$, $p < 0.005$), not different from the PZI or insulin infused rats ($F = 0.308$, n.s.; $F = 2.279$, n.s.) and was significantly lower than the normal rats ($F = 6.79$, $p < 0.05$).

I.A.1.viii) Mean Transit Time (Refer to Figure 9)

The mean transit time (MTT) is a measure of the amount of time a specific label spends in the system. In all cases, of course, the MTT for the 3-H label (MTT3H) is shorter than that of the 14-C label (MTT14C). This is because the latter is able to recyle into glucose and the former is not. In normal rats, the MTT3H is 51 +/- 4 minutes, and the MTT14C is 70 +/- 6 minutes. As one would expect, the increase in insulin titer caused a decrease in the MTT, as an increase in insulin greatly increases the

FIGURE 9: The Mean Transit Time of 3-H-3-Glucose (MTT3H) and of 14-C-U-glucose (MTT14C) in Normal, PZI-Treated (3U), Hypoglycemic Insulin Infused (12-15 mU/kg.min), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infused and 32-36 mg/kg.min glucose infused), Phlorizin Infused (50 ug/kg.min), and Diabetic Rats. Ordinate is in minutes (min). Standard errors of mean are shown as vertical bars. (Exceptions: Normal Rats - MTT14C = 70 +/- 6 min; Diabetic Rats - MTT3H = 156 +/- 44 min, MTT14C = 211 +/- 50 min).

Legend:

NRM = Normal Rats

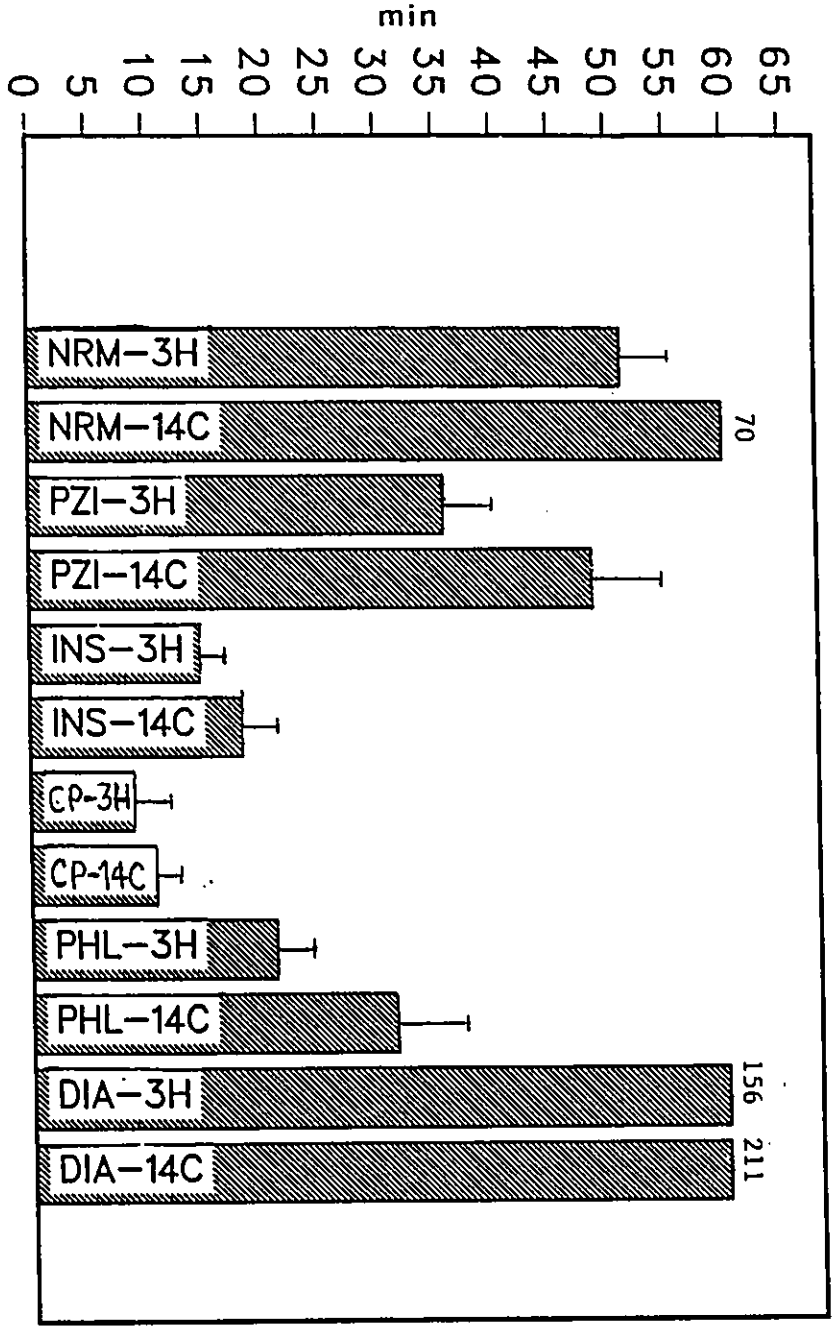
PZI = PZI-Treated Rats

INS = Hypoglycemic Insulin Infused Rats

CP = Euglycemic Insulin Infused Rats

PHL = Phlorizin Infused Rats

DIA = Diabetic Rats



MEAN TRANSIT TIME

plasma clearance rate ($r = 0.98$). The normal MTT are therefore, significantly longer than that in PZI treated rats (MTT3H = 36 +/- 4 min, MTT14C = 48 +/- 6 min) ($F = 50.79$, $p < 0.05$; $F = 20.91$, $p < 0.01$) or in insulin infused rats (MTT3H = 14 +/- 2 min, MTT14C = 17 +/- 3 min) ($F = 119.3$, $p < 0.001$; $F = 98.23$, $p < 0.001$). The shortest MTT was found in hyperinsulinemic euglycemic rats. Their MTT3H was 8 +/- 3 minutes and the MTT14C was 10 +/- 2 minutes. This is significantly shorter than that in any group ($F = 59.45$, $p < 0.001$), except the insulin infused hypoglycemic rats ($F = 0.894$, n.s.). The phlorizin infused rats also had a grossly lowered MTT. Their MTT3H was 22 +/- 3 min and the MTT14C was 30 +/- 6 min. This was significantly lower than in all groups ($F = 34.44$, $P < 0.05$; $F = 20.91$, $p < 0.01$) except the insulin infused rats. The longest MTT of all the groups was seen in the diabetic rats. Their MTT3H was 156 +/- 44 minutes and the MTT14C was 211 +/- 50 minutes. These values were significantly higher than that of all other groups ($F = 69.89$, $p < 0.001$; $F = 155.9$, $p < 0.005$).

I.A.2) Methodological Studies

I.A.2.i) Normal Rats: A/A vs. A/V Designs

(Refer to Table 1)

These studies were carried out to find out whether varying the sites of sampling and tracer injection would affect the kinetic parameters. None of the calculated

parameters differed significantly between these two groups, except that the turnover rate (Rt) was higher by 7% in the A/A (Arterial injection of tracer followed by Arterial sampling of blood) (7.43 +/- 0.15 mg/kg.min) than in the A/V (Arterial injection of tracer followed by Venous sampling of blood) (6.91 +/- 0.26 mg/kg.min) group ($t = 2.704$, $p < 0.05$). It should be noted, however, that the A/A group consisted of 16 rats, whereas, the A/V group had only 4 rats. Nevertheless, a significant difference in the Rt does exist between the groups.

I.A.2.ii) Clamp-Rats: AVA, VAV, VVA, AAV Designs (Refer to Table 2)

These studies were carried out to determine whether, under conditions in which the turnover of glucose is very high (i.e., hyperinsulinemia), varying the sites of tracer administration and sampling would affect kinetic parameters. Of all the calculated parameters, only one (Rt) was found to vary significantly under the four varying conditions. The AVA approach overestimated the glucose infusion rate (34 mg/kg.min) by 11%, whereas the VAV approach gave an accurate estimate of the glucose infusion rate 32.2 mg/kg.min). Both the VVA and AAV approaches gave fairly accurate estimates of the infusion rates, and they did not vary significantly ($t = 1.309$, n.s.). It appeared, therefore, that when there is equivalent tracer supply (i.e., tracer and tracee entering into the same port), it does not matter whether the A/V or V/A technique is used.

Table I

The Calculated Turnover Rate (Rt) in Normal Rats

A/A vs. A/V Design

APPROACH	CALCULATED Rt (mg/kg.min)
A/A	7.43 +/- 0.15
A/V	6.91 +/- 0.26

Mean +/- S.E.M. are shown. A/A > A/V ($p < 0.05$).

Note: A/A = Arterial Injection of Tracer, Arterial Sampling

A/V = Arterial Injection of Tracer, Venous Sampling

Table II

The Calculated Turnover Rate (Rt) in Euglycemic Clamped Rats

AVA vs. VAV vs. VVA vs. AAV Designs

APPROACH	RATE OF TRACEE INFUSION (Rinf) (mg/kg.min)	CALCULATED TURNOVER RATE (Rt) (mg/kg.min)	Rt/Rinf
AVA	34.0	37.9 +/- 1.3	1.11 +/- 0.04
VAV	32.3	32.2 +/- 0.1	0.99 +/- 0.003
VVA	36.0	39.6 +/- 2.3	1.10 +/- 0.06
AAV	36.0	36.3 +/- 1.8	1.01 +/- 0.05

Means +/- S.E.M. are shown.

Note: AVA = Artery tracer injection,
Vein tracee infusion,
Artery sampling.

VAV = Vein tracer, Artery tracee, Vein sampling

VVA = Vein tracer, Vein tracee, Artery sampling

AAV = Artery tracer, Artery tracee, Vein sampling

I.B. COMPARTMENTAL ANALYSIS:

The generalized two-compartment model as put forth by Norwich (1977) was employed. In this model the first compartment is a rapidly mixing compartment, where tracer and tracee glucose mix rapidly, and the second compartment represents the tissues where glucose could distribute itself more slowly. Glucose could be metabolized in both compartments. Plasma samples are withdrawn from the first compartment (sampling compartment). This is the compartment which we are able to manipulate via external mediators (e.g., infused insulin). Further, it is assumed that endogenous glucose produced in the liver enters the first compartment. The rate constants K21 and K12 (K21 = the rate of transfer of glucose from compartment 1 into compartment 2; K12 = the rate of transfer of glucose from compartment 2 into compartment 1) represent the fraction of the glucose mass found in each compartment that is exchanged between the mixing and the tissue compartments per minute. The rates K01 and K02 represent irreversible loss of glucose from the respective compartment due to metabolism and excretion. (K01 = the rate of irreversible loss of glucose from compartment 1; K02 = the rate of irreverisble loss of glucose from compartment 2). Note that we assume that K01 = K02 as per Norwich (1977). In the K01 clamp calculations, we set K01 equal to that of normal rats, and calculated K12, K12 and K02 based on this "clamped" K01 for all experimental groups. We attempted to look at the effect of insulin (in the presence or absence of hypoglycemia), diabetes and

phlorizin on the compartmental rate constants and on the sizes of the compartments.

It should be stated here that in the normal rat, two three-compartment models (a mammillary and a catenary model) were evaluated. Both of these models were found to collapse into the general two-compartmented model upon evaluation.

I.B.1) Volumes of Distribution (Refer to Table 3 and Figures 10 and 11)

Using compartmental analysis the volume of the first compartment (V1) did not differ significantly between any of the groups ($F = 2.139$, n.s.). The size of V1 ranged from 153 +/- 12 ml/kg in the phlorizin infused group to 174 +/- 15 in the insulin infused group. It is clear, therefore, that the size of V1 is quite robust, remaining stable at about 165 ml/kg under various conditions of insulinemia (i.e., normal - V1 = 164 +/- 8 ml/kg, diabetic - V1 = 156 +/- 16 ml/kg, PZI-treated - V1 = 154 +/- 8 ml/kg, glucose clamp - V1 = 166 +/- 17 ml/kg), with or without hypoglycemia.

The size of the second compartment was not as stable as the first. V2 was not significantly different in normal (156 +/- 13 ml/kg), diabetic (144 +/- 26 ml/kg), phlorizin infused (219 +/- 20ml/kg), or PZI-treated (140 +/- 22 ml/kg) rats ($F = 1.531$, n.s.) from V1. Gross hyperinsulinemia, however, in the presence or absence of hypoglycemia caused a marked increase in the size of the

second compartment. In insulin infused rats V2 = 382 +/- 102 ml/kg. In glucose clamp rats, V2 = 331 +/- 113 ml/kg. The size of V2 in these two groups was significantly higher than that of the other four groups ($F = 98.96$, $p < 0.001$, $F = 136.76$, $p < 0.005$), but did not differ amongst themselves ($F = 2.779$, n.s.). Therefore, gross, and mild hyperinsulinemia, irrespective of plasma glucose causes a marked increase in the size of the second compartment. V2 is calculated assuming Vt = V1+V2=((1+(K21/(K02+K12))xV1. In this calculation, it is assumed that C1 equals C2. It should be noted, however, that it is possible to calculate C2 if we have V1 and Vt, or calculate Vt (and V2) if we have C2, but neither V2 nor C2 can be calculated independent of the other from the data available.

TABLE III

Compartmental: Glucose Concentrations, Glucose Masses, Volumes, and Rate Constants in Normal, PZI-Treated (3U Injection), Hypoglycemic Insulin Infused (12-15 mU/kg.min insulin infused), Euglycemic Insulin Infused (12-15 mU/kg.min insulin infused and 32-36 mg/kg.min glucose infused), Phlorizin Infused (50 ug/kg.min), and Diabetic Rats.

Legend:

C1 (=C2) = Concentration of glucose in the first compartment

V1 = Volume of cpt. 1; V2 = volume of cpt. 2

K21 = rate of transfer of glucose from cpt.1 to cpt.2

K12 = rate of transfer of glucose from cpt.2 to cpt.1

K21/K12 = ratio of glucose transfer rates 1-->2 / 2-->1

K01 (=K02) = rate of irreversible loss of glucose from compartments 1 and 2.

K01 Clamp

K02* = rate of irreversible loss of glucose from cpt.2 (with K01 = 0.0183 1/min = K01 for normal rats.)

K21* = K21 calculated with K01 = 0.0183 1/min

K12* = K12 calculated with K01 = 0.0183 1/min

NORMAL = Normal Rats

PZI = PZI-Treated Rats

DIAB = Diabetic Rats

PHL = Phlorizin Infused Rats

INSULIN = Hypoglyccemic Insulin Infused Rats

CLAMP = Euglycemic Insulin Infused Rats

	NORMAL	PZI	DIAB	PHL	INSULIN	CLAMP
C1 (=C2)	132	140	613	76	48	118
	+/-	+/-	+/-	+/-	+/-	+/-
(mg/dl)	3	7	93	2	4	13
V1	164	154	156	153	174	166
	+/-	+/-	+/-	+/-	+/-	+/-
(ml/kg)	8	8	16	12	15	17
V2	156	140	195	219	382	331
	+/-	+/-	+/-	+/-	+/-	+/-
(ml/kg)	13	22	26	20	102	113
M1	214	215	955	116	83	196
	+/-	+/-	+/-	+/-	+/-	+/-
(mg/kg)	7	11	53	10	7	18
M2	204	196	1195	166	183	391
	+/-	+/-	+/-	+/-	+/-	+/-
(mg/kg)	19	31	308	22	74	122
K21	0.1393	0.1231	0.252	0.2376	0.2095	0.2655
	+/-	+/-	+/-	+/-	+/-	+/-
(1/min)	0.02	0.016	0.05	0.03	0.032	0.041
K12	0.1284	0.1151	0.195	0.1275	0.0471	0.0582
	+/-	+/-	+/-	+/-	+/-	+/-
(1/min)	0.012	0.017	0.029	0.007	0.003	0.004
K21/K12	1.08	1.13	1.29	1.86	4.45	4.56
	+/-	+/-	+/-	+/-	+/-	+/-
	0.19	0.09	0.31	0.26	0.74	0.79
K01-K02	0.0183	0.0254	0.0063	0.0382	0.0483	0.0749
	+/-	+/-	+/-	+/-	+/-	+/-
(1/min)	0.00005	0.0009	0.0008	0.002	0.004	0.0005
K02*	0.0182	0.0315	-0.0004	0.0474	0.0528	0.0844
	+/-	+/-	+/-	+/-	+/-	+/-
(1/min)	0.0007	0.001	0.0009	0.0009	0.0009	0.01
K21*	0.1431	0.1521	0.2359	0.2455	0.2385	0.3107
	+/-	+/-	+/-	+/-	+/-	+/-
(1/min)	0.022	0.026	0.058	0.041	0.038	0.05
K12*	0.1265	0.1222	0.2088	0.1043	0.0417	0.0431
	+/-	+/-	+/-	+/-	+/-	+/-
	0.018	0.021	0.029	0.019	0.009	0.009

Mean +/- S.E.M. are shown.

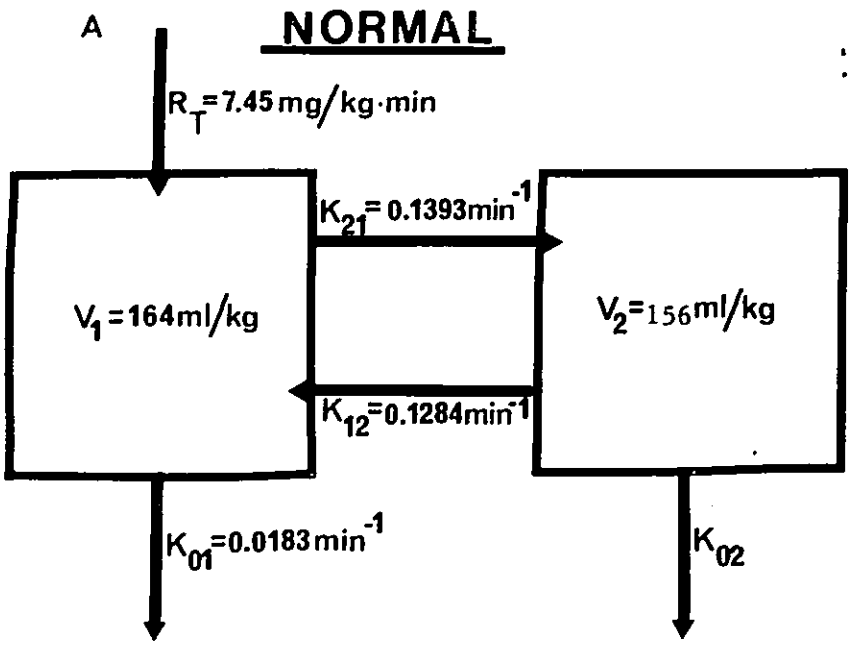
FIGURE 10: Two compartmental analysis of the glucose system. Rt is the turnover rate (or hepatic glucose production rate) and has units mg/kg min. V1 is the volume of the first compartment and has units ml/kg. V2 is the volume of the second compartment and has units ml/kg. K21 is the rate of transfer of glucose from the first compartment into the second compartment and has units 1/min. K12 is the rate of transfer of glucose from the second compartment into the first compartment and has units 1/min. K01 is the rate of irreversible loss of glucose from the first compartment and has units 1/min. K02 is the rate of irreversible loss of glucose from the second compartment and is equal to K01.

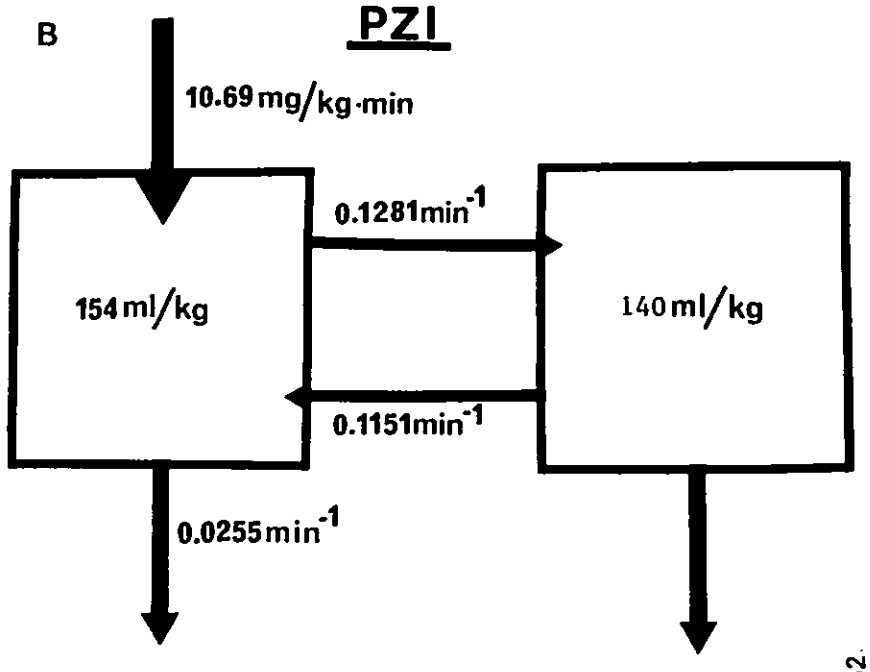
Figure 10(a) represents Normal Rats

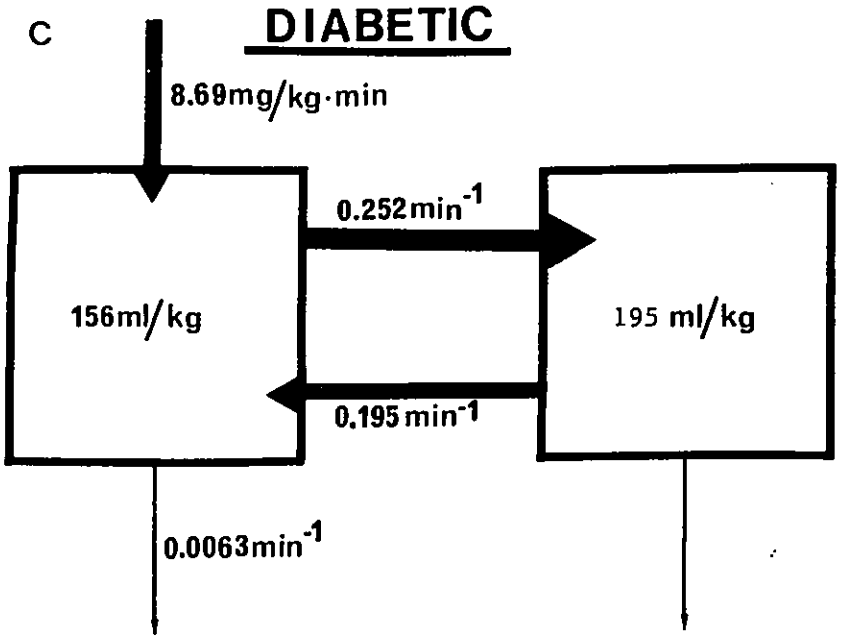
Figure 10(b) represents PZI-Treated Rats

Figure 10(c) represents Diabetic Rats

Figure 10(d) represents Phlorizin Infused Rats


A
NORMAL
R_T=7.45 mg/kg·min
K_{21}= 0.1393min^{-1}
V_1 =164ml/kg
V_2=156ml/kg
K_{12}=0.1284min^{-1}
K_{01}=0.0183 min^{-1}
K_{02}


B
PZI
10.69 mg/kg·min
0.1281min^{-1}
154 ml/kg
140 ml/kg
0.1151min^{-1}
0.0255 min^{-1}


C
DIABETIC
8.69mg/kg·min
0.252 min^{-1}
156ml/kg
195 ml/kg
0.195 min^{-1}
0.0063min^{-1}

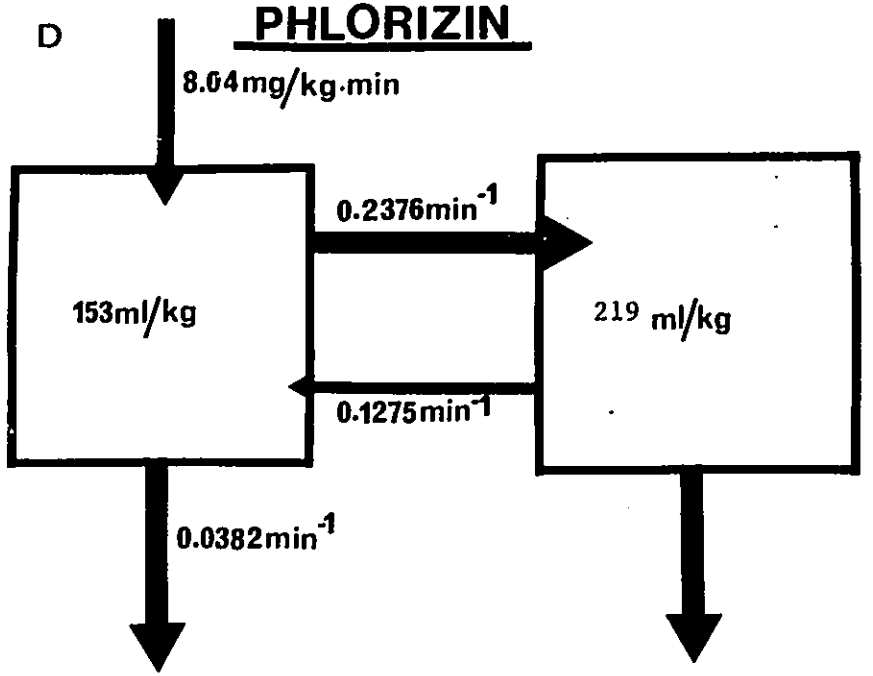

D
PHLORIZIN
8.04mg/kg·min
0.2376min^{-1}
153ml/kg
219 ml/kg
0.1275min^{-1}
0.0382 min^{-1}

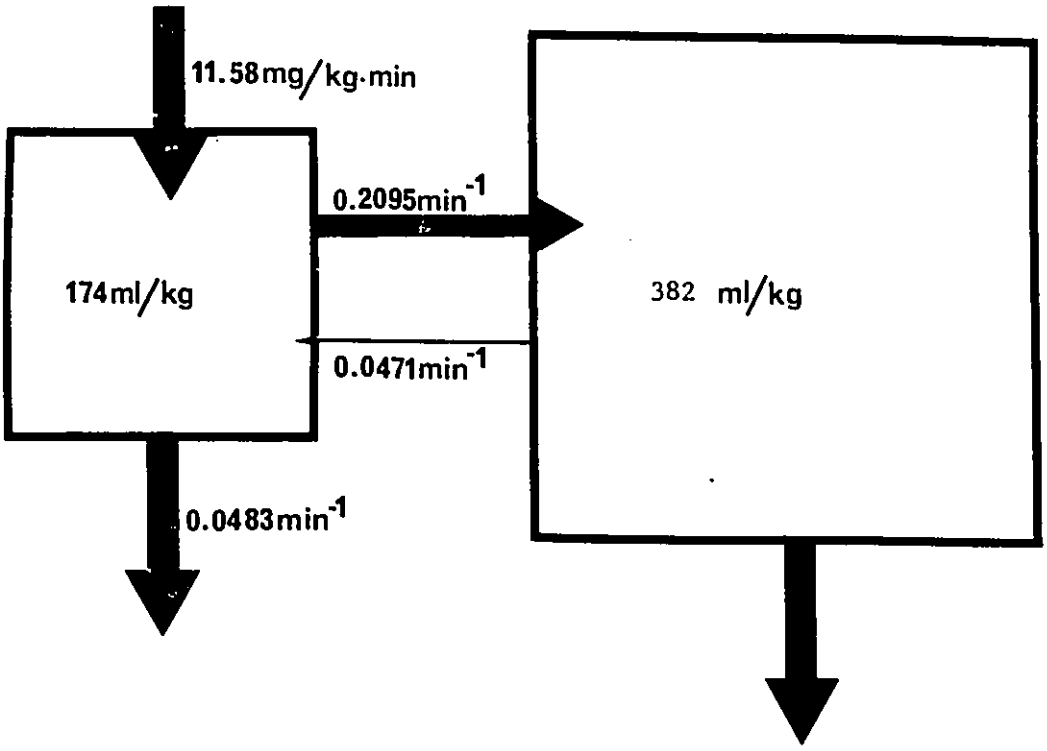
FIGURE 11: Two compartmental analysis of the glucose system. Rt is the turnover rate (or hepatic glucose production rate) and has units mg/kg min. V1 is the volume of the first compartment and has units ml/kg. V2 is the volume of the second compartment and has units ml/kg. K21 is the rate of transfer of glucose from the first compartment into the second compartment and has units 1/min. K12 is the rate of transfer fo glucose from the second compartment into the first compartment and has units 1/min. K01 is the rate of irreversible loss of glucose from the first compartment and has units 1/min. K02 is the rate of irreversible loss of glucose from the second compartment and is equal to K01.

Figure 11(a) represents Hypoglycemic Insulin Infused Rats

Figure 11(b) represents Euglycemic Insulin Infused Rats

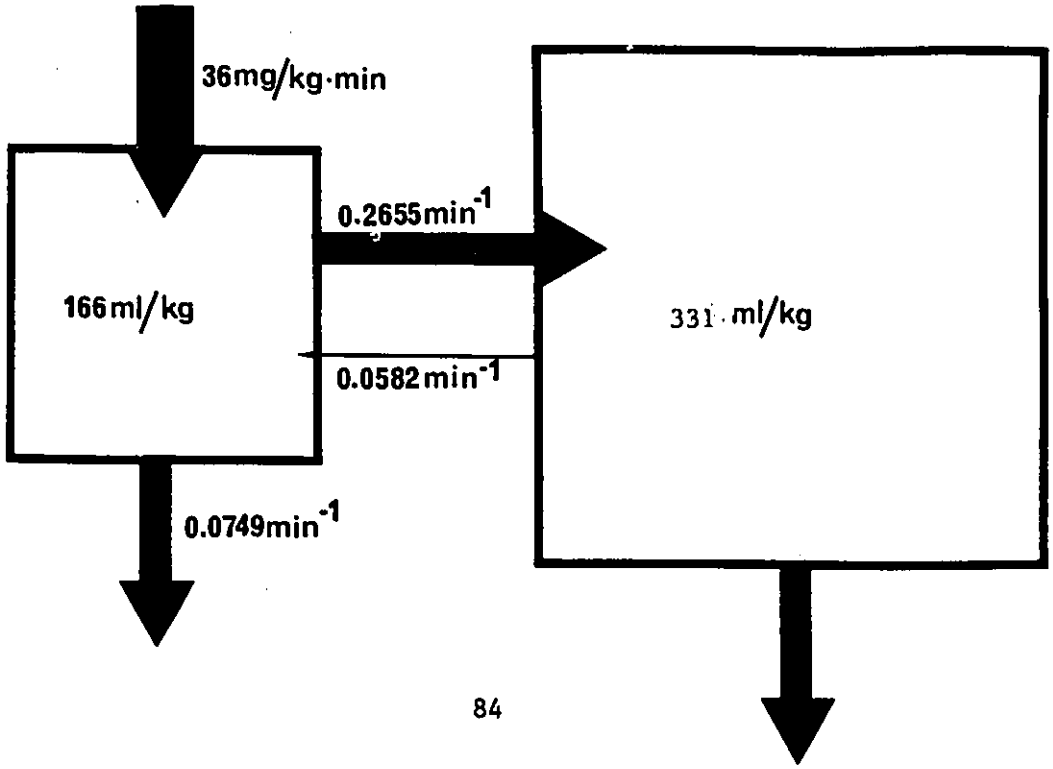
A

INSULIN



B

CLAMP



I.B.2. Rate Constants

(Refer to Table 3, and Figures 10 and 11)

The rate constants between the two compartments (K21 and K12), and the rate constants of irreversible loss of glucose from the two compartments by metabolism and excretion (K01 - K02) are affected by the plasma IRI level. K02*, K12*, and K21* were calculated by clamping the value of K01 to that in normal rats.

In normal rats K21 (0.1393 +/- 0.02 min^{-1}) is not different from K12 (0.1284 +/- 0.012 min^{-1}) ($t = 0.498$, n.s.). The ratio of K21/K12 equals 1.08 +/- 0.19. In PZI rats, K21 (0.1281 +/- 0.016 min^{-1}) and K12 (0.1151 +/- 0.017 $min.^{-1}$) are the same as in normal rats ($F = 1.109$, n.s.; $F = 0.776$, n.s.). For PZI rats the ratio of K21/K12 equals 1.13 +/- 0.9. Mild hyperinsulinemia in the PZI rats caused the rates of irreversible loss of glucose from the two compartments, K01 (=K02), to be increased significantly over the normal values ($F = 67.68$, $p < 0.005$). K01 (=K02) = 0.0254 +/- 0.0009 min^{-1} in the PZI treated rats, which is an increase of 39% over the normal values of K01 (=K02) (0.0183 +/- .00005 min^{-1}).

In diabetic rats K21 was by 81% above over baseline: 0.252 +/- 0.050 min^{-1} ($F = 187.5$, $p < 0.001$). K12 also

increased by 52% over baseline to 0.195 +/- 0.029 min^{-1} (F = 113.44, $p < 0.001$). The ratio of K21/K12 is 1.29 +/- 0.31 in diabetes is however, not different from normal and PZI rats (F = 2.223, n.s.). K01 (=K02) (0.0063 +/- 0.0008 min^{-1}) in diabetic rats is significantly lower than K01 (=K02) in both normal (F = 16.69, $p < 0.01$) and in PZI (F = 37.98, $p < 0.05$) rats.

In phlorizin infused rats, a significant increase in K21 = 0.2376 +/- 0.030 min^{-1} and K01 (=K02) = 0.0382 +/- 0.002 min^{-1}) over normal (F = 35.55, $p < 0.05$; F = 50.91, $p < 0.005$) was observed. The K21/K12 ratio (1.86 +/- 0.26) in the phlorizin infused rats, indicates that the rate of clearance of glucose from the first compartment is significantly larger than in normal rats. The K12 = 0.1275 +/- 0.007 min^{-1}) in phlorizin infused rats does not differ from normal (F = 0.989, n.s.).

Gross hyperinsulinemia, irrespective of the level of plasma glucose has major effects on the compartmental rate constants. K21 (0.2095 +/- 0.032 min^{-1}) in insulin infused rats is increased 50% over baseline (F = 67.98, $p < 0.005$), and K21 (0.2655 +/- 0.041 min^{-1}) is increased by 90% over baseline (F = 134.55, $p < 0.001$) in glucose clamp rats. This magnitude of increase in K21 is similar to that seen in the diabetes and in the phlorizin infused rats. K12 (0.0471 +/- 0.003 min^{-1}) in insulin infused rats is decreased by 63% from baseline (F = 22.33, $p < 0.01$), and K12 (0.0582 +/- 0.004 min^{-1}) is decreased by 55% from baseline (F = 77.87, p

< 0.005) in glucose clamp rats. This indicates that gross hyperinsulinemia irrespective of the plasma glucose concentration causes the second compartment to quasi act as a sink for glucose. The magnitude of this sink effect can be shown by the fact that the ratio of K21/K12 in insulin infused rats is 4.45 +/- 0.74, and 4.56 +/- 0.79 when plasma glucose was clamped. The irreversible loss of glucose from the compartments as indicated by K01 (-K02) is also grossly elevated above baseline in all of the insulin infused rats. K01 (-K02) = 0.0483 +/- 0.004 min^{-1} is significantly greater than normal rats ($F = 87.45$, $p < 0.001$). Similarly, when plasma glucose is kept steady at the euglycemic level K01 (-K02) = 0.0749 +/- 0.009 min^{-1} is also significantly greater than normal ($F = 202.6$, $p < 0.005$).

We clamped the value of K01 at the value of the rate of irreversible loss of glucose from the first compartment as seen in the normal rats group. The rationale behind this procedure was that, the first compartment appeared to behave almost identically in all of the different experimental groups. The size of the first compartment is very robust. On that note, we assumed that the first compartment may simply be a mixing compartment, with very little metabolism. Under this assumption, therefore, we could assume also, that the irreversible rate of loss of glucose from this first (primarily mixing) compartment was equal in all groups. Using these assumptions we found that the K21* did not differ from the K21 in any of the groups ($F = 1.101$, $p <$

0.05) and K12* did not differ from K12 in any of the groups ($F = 0.991$, $p < 0.05$). The value of K02* in normal rats was equal to K01 (=K02) ($F = 69.76$, $p < 0.001$). This was not only expected, but also indicated to us that our "K01 clamp" calculations were correct. K02* was seen to increase significantly over K01 (=K02) in PZI-treated ($F = 21.13$, $p < 0.005$), in euglycemic clamp ($F = 9.65$, $p < 0.01$), and in phlorizin infused rats ($F = 15.77$, $p < 0.01$). K02* was not significantly different from K01 (=K02) in insulin infused rats ($F = 1.344$, n.s.). In diabetic rats, the K02* was significantly decreased from K01 (=K02) ($F = 7.34$, $p < 0.005$) and equalled zero.

I.B.3 Compartmental Glucose Concentrations and Masses

(Refer to Table 3, and Figures 10 and 11)

The concentration of glucose in the first compartment (C1) was assumed to equal the plasma glucose concentration as the first compartment since plasma forms a part of this compartment by definition. The concentration of glucose in the second compartment, C2, was assumed to equal C1 (diStefano, 1982).

Because of the stability in the size of the first compartment (i.e., V1 stable), the actual mass of glucose in the first compartment (M1) varied within the different experimental groups as a linear function of plasma glucose concentration. In diabetes, M1 (955 +/- 53 mg/kg) was

concentration. In diabetes, M1 (955 +/- 53 mg/kg) was significantly greater than all other groups ($F = 34.45$, $p < 0.001$). M1 was the same in normal (214 +/- 7 mg/kg), PZI-treated (215 +/- 11 mg/kg), and during a glucose clamp (196 +/- 18 mg/kg), as the plasma glucose in these three groups was about the same ($F = 3.112$, n.s.). M1 was decreased by 46% in phlorizin infused rats (116 +/- 10 mg/kg) ($F = 67.67$, $p < 0.005$) and by 61% in hypoglycemic insulin infused rats (83 +/- 7 mg/kg) ($F = 30.21$, $p < 0.001$).

The mass of glucose in the second compartment (M2) was largest in diabetic rats (1195 +/- 308 mg/kg) ($F = 301.1$, $p < 0.001$). M2 was the same in normal (204 +/- 19 mg/kg) and PZI-treated (196 +/- 31 mg/kg) rats as M1 ($F = 2.556$, n.s.). M2 was greater than M1 by 30% (166 +/- 22 mg/kg) in the phlorizin treated rats, by 120% (183 +/- 74 mg/kg) in insulin infused rats and by 99% (391 +/- 122 mg/kg) in clamp rats.

I.C.) Non-Compartmental total volume of distrubution (Vt-NCA) vs. Compartmental total volume of distribution (Vt-CA) (Refer to Table 4)

In normal rats (Vt-NCA = 294 +/- 20 ml/kg, Vt-CA = 320 +/- 30 ml/kg; $t = 1.24$, n.s.), PZI-treated rats (Vt-NCA = 269 +/- 19 ml/kg, Vt-CA = 294 +/- 45 ml/kg; $t = 0.89$, n.s.), and phlorizin infused rats (Vt-NCA = 287 +/- 34 ml/kg, Vt-CA = 372 +/- 45 ml/kg; $t = 1.05$, n.s.) the volume of distribution as calculated by the non-compartmental approach

(Vt-NCA) is not different from the volume of distribution as calculated by the compartmental approach (Vt-CA). The increase in hyperinsulinemic rats is only revealed by compartmental analysis. The increment over the respective Vt-NCA's are significant in the hypoglycemic insulin infused group (65%: Vt-NCA = 337 +/- 31 ml/kg, Vt-CA = 556 +/- 150 ml/kg; $t = 5.11$, $p < 0.02$) and in the euglycemic insulin infused group (64%: Vt-NCA = 303 +/- 40 ml/kg, Vt-CA = 497 +/- 119 ml/kg; $t = 3.37$, $p < 0.01$).

Table IV

Total Volume of Distribution as calculated using the Non-Compartmental Approach (Vt-NCA) vs. the Total Volume of Distribution as calculated using the Compartmental Approach (Vt-CA).

GROUP	Vt-NCA (ml/kg)	Vt-CA (ml/kg)	t	
NORMAL	294 +/- 20	320 +/- 30	1.24	n.s.
PZI	269 +/- 19	294 +/- 45	0.89	n.s.
INSULIN INFUSION	337 +/- 31	556 +/- 150	5.11	$p < 0.02$
GLUCOSE CLAMP	303 +/- 40	497 +/- 119	3.37	$p < 0.01$
PHLORIZIN INFUSION	287 +/- 34	372 +/- 45	1.32	n.s.
DIABETES	243 +/- 44	351 +/- 63	0.51	n.s.

Mean +/- S.E.M. are shown.

Note: For the calculation of the Vt-CA and Vt-NCA refer to the "Calculations" section.

II) EXPERIMENTS OUT OF DYNAMIC STEADY STATE (ODSS)

II.A) ODSS Caused by an Insulin Infusion (15 mU/kg.min)

(Refer to Figure 12)

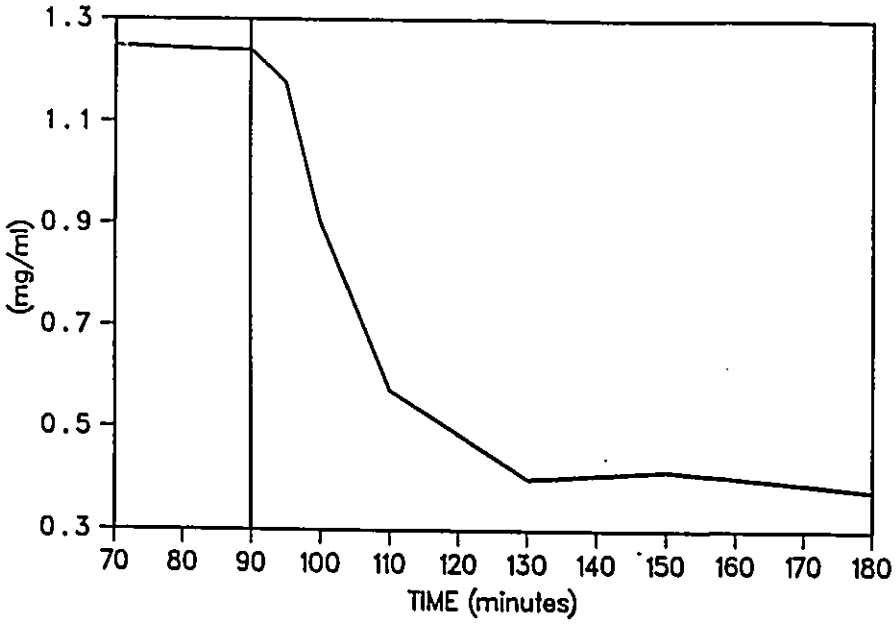
The infusion of 15 mU/kg.min of crystalline insulin caused the plasma glucose concentration to drop by 66% from a baseline of 1.25 +/- 0.04 mg/ml to 0.42 +/- 0.11 mg/ml ($t = 2.645$, $p < 0.001$). This hypoglycemia caused by the insulin infusion resulted in an immediate increase (peaking at 30 minutes after the beginning of the insulin infusion, or $t = 120$ minutes) in the hepatic glucose output (Ra). Figure 12 shows Ra calculated by four different methods. (A) Calculations based on a two compartment model: i) using the insulin 2-compartment model K's and V's from DSS; ii) using the normal 2-compartment model K's and V's from DSS. B) Calculations based on a single compartment model: i) using the pool fraction (p) set equal to 0.5 in Steele's equation; ii) using pV equal to Vl = 165 ml/kg in Steele's equation. From the first set of calcuations it can be seen that with the K's and V's obtained from the DSS model in the insulin infused rats, Ra was calculated to increase 328% from the baseline of 7.56 +/- 0.11 mg/kg.min to 32.41 +/- 2.14 mg/kg.min ($t = 8.98$, $p < 0.001$). Using the K's and V's obtained from the DSS model in normal rats the Ra was estimated to increase by 205% over the baseline to 23.07 +/- 1.62 mg/kg.min ($t = 6.77$, $p < 0.005$). The Ra calculated with K's and V's for insulin infused rats is therefore significantly different from the Ra obtained from the K's and V's found in normal rats in the p.a. state ($t = 3.12$, p

< 0.01). From the one-compartment-based calculations (using Steele's equation), with p = 0.5, the Ra was seen to increase by 170% over baseline to 20.4 +/- 1.19 mg/kg.min (t = 7.13, p < 0.001) . Using pV = 165 ml/kg, calculated the resultant increase in the Ra was increased by 159% over baseline to 19.6 +/- 1.41 mg/kg.min (t = 8.22, p < 0.001). The two approximations of the magnitude of increase in the Ra due to the insulin infusion based on single compartment models were not significantly different (t = 1.004, n.s.). It should be noted that the peak Ra was attained 30 minutes (t = 120 minutes) into the insulin infusion in all four cases. By t = 180 minutes, all four approximations of the Ra levelled off at about 12 mg/kg.min as DSS had been attained.

Both two-compartmental and both one-compartmental approximations of the increase in Ra due to the infusion of insulin were significantly greater than normal. We are however, not completely confident about our two compartment approximations. The use of the normal Kij's and V's from DSS for the calculation of Ra ODSS due to the infusion of insulin is not completely correct. The normal K's and V's are indicative of an animal that is not insulinized at all. Conversely, the use of the insulin K's and V's from DSS for the ODSS calculations of Ra are for the grossly hyperinsulinemic animal. In our DSS calculations, however, we found that the first compartment was very robust in size. We therefore feel more confident with the Ra estimation using the pV = 165 ml/kg for the insulin induced ODSS condition.

FIGURE 12: Effect of infusing insulin at 15 mU/kg.min into normal rats on plasma glucose concentration (mg/ml) and rate of glucose appearance (Ra) (mg/kg.min). Ra is calculated using both the two compartment approximation (i.e., insulin K's and V's, and normal K's and V's) and the one compartment approximation (Steele's equation) (i.e., p = 0.5, and pV = 165 ml/kg) Abciss time in minutes. Ordinate: Plasma glucose is measured in mg/ml; Ra is measured in mg/kg.min.

PLASMA GLUCOSE
INSULIN ODSS



INSULIN INFUSION - ODSS

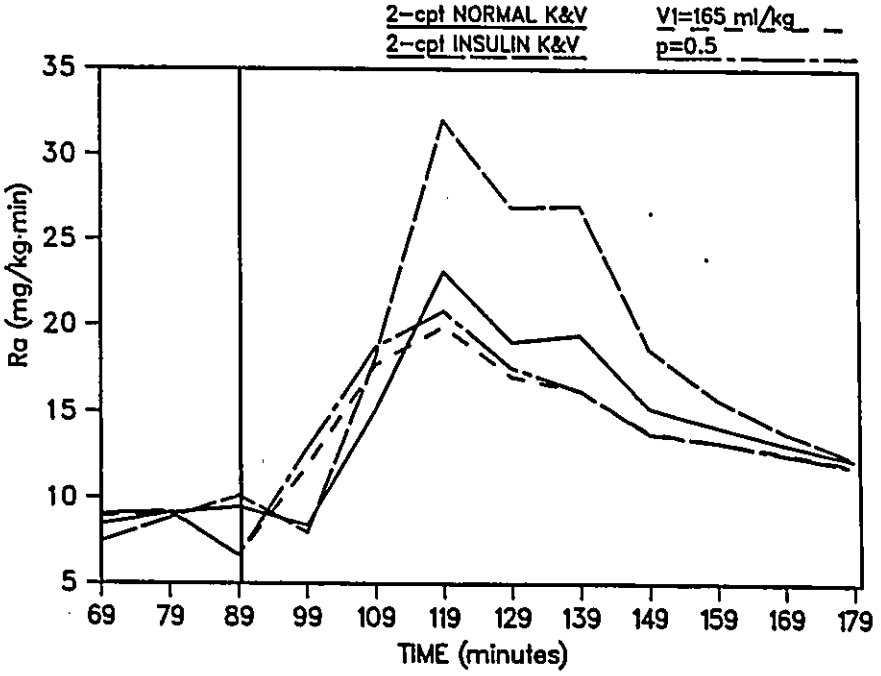
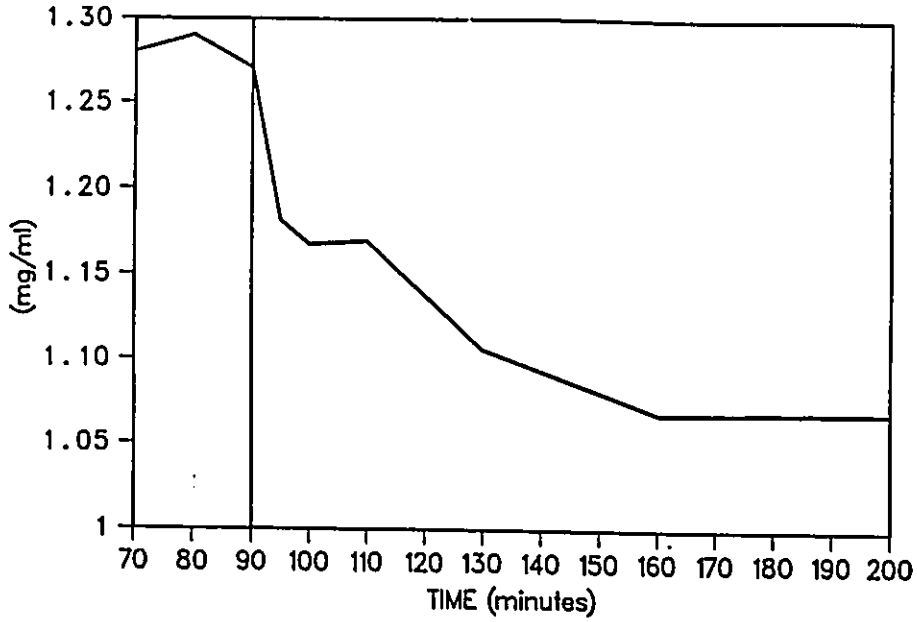
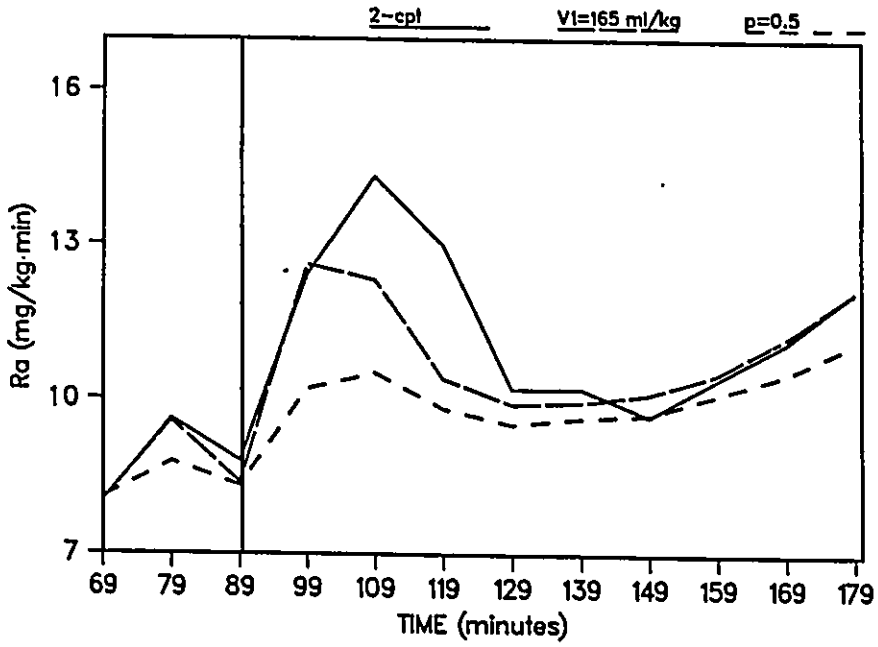


FIGURE 13: Effect of infusing phlorizin at 50 ug/kg.min into normal rats on plasma glucose concentration (mg/ml) and rate of glucose appearance (Ra) (mg/kg.min). Ra is calculated using both the two compartment approximation (i.e., phlorizin K's and V's) and the one compartment approximation (Steele's equation) (i.e., p = 0.5, and pV = 165 ml/kg). Abcissa: time in minutes. Ordinate: Plasma glucose is measured in mg/ml; Ra is measured in mg/kg.min.

PLASMA GLUCOSE
PHLORIZIN ODSS



PHLORIZIN INFUSION - ODSS



II.B ODSS Caused by a Phlorizin Infusion (50 ug/kg.min)

(Refer to Figure 13)

. The infusion of 50 ug/kg.min of phlorizin caused the plasma glucose to decrease by 19% from 1.27 +/- 0.06 mg/ml to 1.06 +/- 0.04 mg/ml ($t = 2.251$, $p < 0.10$). This mild hypoglycemia caused only a small increase in the Ra. The Ra was calculated by three methods: A) Two-compartment calculations using the K's and V's from the phlorizin infused rats in DSS; One-compartment calculations; i) $p = 0.5$ in Steele's equation, ii) $pV = 165$ ml/kg in Steele's equation). Using the two compartment approximation the Ra was seen to increase by 70% over the baseline from 8.72 +/- 1.09 mg/kg.min to 14.91 +/- 3.87 mg/kg.min ($t = 3.058$, $p < 0.05$). Using $p = 0.5$ in Steele's equation, the Ra was seen to increase to 10.39 +/- 1.79 mg/kg.min ($t = 0.989$, n.s.). Using the $pV = 165$ ml/kg, the Ra was seen to increase to 12.71 +/- 3.24 mg/kg.min ($t = 1.233$, n.s.).

Despite the significant increase in Ra due to an infusion of phlorizin as calculated by the two-compartmental approximation using the K's and V's from the fully phlorizinized animal from DSS, neither of the one-compartment approximations gave a significant increase in the Ra for the ODSS situation. Again, as in the insulin infused ODSS calculations, we are not confident about the two-compartment caluclations, as the K's and V's from the

fully phlorizined aminal in DSS is not applicable to the ODSS situation. We therefore, are more confident with the pV - 165 ml/kg approximation of the Ra using Steele's equation. From this, we conclude that rats do not have a very good non-hypoglycemic glucoregulatory mechanism.

CHAPTER V

DISCUSSION

I) EXPERIMENTS IN DYNAMIC STEADY STATE (DSS)

In the Dynamic Steady State condition, a decay curve for the concentration of tracer in the plasma (C*) was fitted with respect to time with the aid of computer software (BMDP-AR, and BMDP-3R). Both double and triple exponential functions were fitted to the data, but it was shown that the former in general gave the best curve as estimated by the smaller variation in the error estimate of the area below the curve. Once the exponential functions were obtained, the data were analyzed using both compartmental and non-compartmental approaches. Both approaches are linear models for the analysis of data obtained in kinetic experiments (Carson et al., 1983). The non-compartmental approach is usually adequate for describing the dynamics of a metabolic system. In this representation the system is treated as a homogeneous entity. For example, the concentration of a metabolite in plasma is implicitly assumed to be the concentration of the metabolite in the system. In other words, the plasma, which in fact contains gradients of concentration within it, is considered to be a single compartment in which the substance is homogeneously distributed (Carson et al., 1983). Furthermore, any endogenously produced or exogenously administered metabolite is assumed to become instantaneously

become homogeneous throughout the system. Clearly, such a representation of any system has its limitations.

Estimations of the kinetic parameters thus are performed using data obtained from the only compartment available for sampling: the blood plasma. From the loss of labelled metabolite from the plasma, therefore, we attempt to predict the kinetic and metabolic processes affecting the metabolite throughout the system. Because we have access to only one pool for sampling, if "de-novo" synthesis and/or irreversible loss of the metabolite occurs in compartments not accessible to direct test inputs and measurements (i.e., $K02 > 0$), then the non-compartmental analysis of the kinetic data can lead to errors (DiStefano, 1982; Cobelli and Toffolo, 1984).

Compartmental modelling is therefore preferred (Cobelli and Toffolo, 1984) in cases where the distribution and the disposition of a metabolite is not homogeneous throughout the system. Simple compartmental models, such as the one proposed by Steele (1959) in which the model of a system is reduced to a single compartment in which by definition tracer mixes instantaneously with any endogenously or exogenously appearing metabolite, have given way to the more complex two and three compartmental systems (Norwich , 1977; Ferrannini et al., 1985). As Riggs (1963) suggested, however, the analysis of multi-compartmental systems requires many more assumptions, as sampling can only be

conducted from the single plasma compartment, from which the parameters governing the other compartments are estimated. Riggs (1963) indicated that, in a system of N compartments, each having pathways both to and from every other compartment as well as a pathway leading out of the system, then, there would be altogether N^2 different pathways each with its own rate constant. Each compartment would also have a value, so that such a system of N compartments may have as many as $N^2 + N$ arbitrary constants or parameters requiring the solution of a set of N simultaneous differential equations, one for each compartment. Clearly, therefore, the greater the number of compartments we try to predict the kinetics of, the greater the number of assumptions that must be made.

A decay function described as a sum of two exponentials indicates that the system must contain at least two compartments. The rapidly decreasing first part of the curve reflects the rapidly mixing compartment in which the tracer glucose mixes in the system. The more slowly decreasing second part of the curve reflects the compartment in which glucose is metabolized. If a large enough number of data points are available in the early part of the curve, it is possible to fit a sum of three exponentials to the tracer decay from the plasma. A sum of three exponentials would indicate, first, a rapidly mixing compartment, followed by at least two metabolizing compartments. All of

our efforts to fit a triple exponential function to our data failed because the coefficient and the exponent of the third exponential did not differ significantly from those of the second exponential. We analyzed our data using both non-compartmental and two and three compartmental approaches. All of the three compartmental systems that were fitted, however, were seen to collapse into a simpler two compartmental representation.

In dynamic steady state conditions, the estimation of the turnover rate (Rt) and the irreversible disposal rate (Ri) of glucose from the system do not differ in either non-compartmental or compartmental approaches. In both cases, the virtual rate of clearance of the substance (tracee) (ml/min) is calculated from the ratio of the amount of injected tracer glucose (M*) by the area under the decay curve. Similarly the turnover rate of glucose (mg/min) and the irreversible disposal rate (mg/min) is calculated from the product of the plasma clearance rate of glucose and the plasma concentration of glucose (mg/ml) (both of which are constant during DSS). The non-compartmental approach also allows us to calculate the mean transit time (min) of the tracer, which is the time the tracer or tracee molecule spends in the entire system. The apparent volume of distribution can be calculated as the product of the clearance rate of glucose and the mean transit time. If, however, glucose does not distribute itself throughout the system instantaneously, as assumed by the non-compartmental

approach, then this procedure is inadequate to completely describe the glucose system (Cobelli and Toffolo, 1984). The different glucose compartments within the system have unique turnover rates (and rate constants). Therefore, these compartments will have unique mean transit times, glucose masses and steady state equivalent distribution volumes, that are affected by definite rates of irreversible losses (Cobelli and Toffolo, 1984). Therefore, the assumptions inherent in the non-compartmental approach do not hold true for the glucose system (Cobelli and Toffolo, 1984, Ferrannini et al., 1985) (That is, glucose is directly utilized in the plasma or is removed by all tissues so rapidly that this process is kinetically indistinguishable from disappearance from plasma).

I.A) Non-Compartmental Analysis

The basal glucose concentration in the plasma of normal control rats in the postabsorptive (p.a.) state was 132 +/- 3 mg/dl and the basal IRI was 12.6 +/- 0.8 uU/ml. These levels were in the range of reported values for both glucose, 90 -138 mg/dl (Hetenyi and Ferrarotto, 1983a; Sonne et al., 1987; Madar, 1986; Proietto et al., 1987), and plasma insulin, 6.5 - 12.5 uU/ml (Kraegen et al., 1983). In the rats treated with PZI, but allowed access to 10% glucose solution overnight, the plasma glucose was 114 +/- 7 mg/dl, and the IRI level was 24 +/- 4 uU/ml. These rats, therefore, regulated against the mild hyperinsulinemia, by

drinking just enough of the glucose solution to maintain euglycemia. In the rats that were infused with 12 - 15 mU/kg.min insulin, the plasma glucose decreased to 48 +/- 4 mg/dl within 45 - 60 minutes, with a correspondingly elevated IRI of 531 +/- 89 uU/ml. Kraegen et al. (1983) reported decreased plasma glucose levels of 42 mg/dl within 2 hours, in rats infused with 1.67 mU/kg.min porcine insulin. In dogs, the plasma glucose has also been shown to decrease to 40 - 45 mg/dl during an infusion of 7 mU/kg.min insulin (Gauthier et al., 1980). In 5 rats, we infused between 1.5 and 2.2 uU/kg.min insulin (during a euglycemic clamp), and the time to reach DSS was about 150 - 180 minutes. In euglycemic hyperinsulinemic rats, the plasma glucose was maintained at 118 +/- 13 mg/dl during an infusion of 12 - 15 mU/kg.min insulin with 34 - 36 mg/kg.min glucose or, when 1.5 - 2.2 mU/kg.min insulin was infused, with 13 - 21 mg/kg.min glucose. The plasma IRI in experiments in which "glucose clamp" was applied was 630 +/- 216 uU/ml. In the rats infused with phlorizin (50 ug/kg.min), the inhibition of glucose reabsorption by the proximal kidney tubules resulted in a decrease in the plasma glucose of 47% below basal to 76 +/- 2 mg/dl after 150 min. Consequently, due to the mild hypoglycemia, a decrease in the IRI to 5.5 +/- 0.2 uU/ml was observed. In dogs, an infusion of phlorizin has been shown to decrease plasma glucose by only 15 - 20 % (Gauthier et al., 1980). In diabetes, the plasma glucose was elevated to 613 +/- 93 mg/dl, and the relative insulin titer in the plasma was not

measurable, and therefore assumed to be virtually zero.

The calculation of the plasma clearance rate (PCR) is model independent. That is, the value of the PCR is identical if calculated using either the non-compartmental or the compartmental approximations. In normal rats, the PCR was calculated as 5.73 +/- 0.12 ml/kg.min. This agrees well with the reported values of 4.81 ml/kg.min (Proietto et al., 1987) and 7.45 ml/kg.min (Sonne et al., 1987). Our results indicated that the PCR increased in parallel to plasma IRI. We did not conduct any experiments to detect for any changes from this linearity. In rats treated with PZI, the PCR rose to 7.63 +/- 0.62 ml/kg.min. Hyerinsulinemic hypoglycemic rats had a PCR of 26.7 +/- 3.1 ml/kg.min, and euglycemic clamped rats had a PCR of 35.2 +/- 4.8 ml/kg.min. The correlation coefficient between plasma IRI and glucose clearance equalled 0.98. This agrees with previously reported studies. Cole et al., (1973) showed that $r = 0.91$ for the clearance of glucose vs. plasma IRI. Vranic et al., (1980) showed that the clearance rate of glucose is directly proportional to the plasma insulin concentration and is independent of the glucose concentration. Radziuk and Lickley (1985) showed that, at a constant insulin concentration, the increase in plasma concentration of glucose caused a linear increase in the utilization of glucise within a wide range of glucose concentrations in the plasma. They showed that this line did not pass through the origin; rather, a positive y-

intercept was noted. This indicated that at even very lwo plasma concentrations, some tissues still utilized glucose. Consequently, in diabetic rats, the plasma clearance rate of glucose was only 1.66 +/- 0.29 ml/kg.min. Increased renal clearance of glucose in the rats infused with phlorizin caused an increase in the PCR of glucose to 10.67 +/- 0.79 ml/kg.min, despite the lower than the basal plasma IRI level.

The turnover rate (Rt) of glucose in DSS is the rate at which glucose is being utilized and produced. The turnover rate is calculated from the decay curve of the 3-H labelled glucose. The irreversible disposal rate (Ri) of glucose is calculated from the 14-C labelled glucose decay curve. The percentage of recycling of glucose carbons from labelled glucose into intermediates and then back into newly synthesized glucose via the Cori and alanine cycles is a fairly good index of the fraction of the turnover rate contributed by gluconeogenesis. It is simply calculated as ((Rt-Ri)/Rt) x 100%. The basal turnover rate of glucose in normal rats was equal to 7.43 +/- 0.15 mg/kg.min. This value falls well within the boundaries of reported turnover rates which range from 6.06 mg/kg.min (Proietto et al., 1987) to 13.8 mg/kg min (Heath and Corney, 1973). Other reported values in the literature are 6.1 mg/kg.min (Hetenyi and Ferrarotto, 1983a), 7.3 mg/kg.min (Sacca et al., 1974), 7.38 mg/kg.min (Sonne et al., 1987) and 9.5 - 12.2 mg/kg.min

(Katz et al., 1981). The basal turnover rate in rats, relative to body weight, is significantly higher than that in man or dog. In man the Rt ranges from 1.40 mg/kg.min (Streja et al., 1977) to 2.39 mg/kg.min (Hall et al., 1979). In dogs, the basal Rt has been shown to range between 2.58 mg/kg.min (Altszuler et al., 1975) and 3.57 mg/kg.min (Issekutz et al., 1974). The percent recirculation equalled 23.5% in the normal rats. This is slightly below the 35-40% recycling estimated by Katz et al. (1974b) and the 35% estimated by Hetenyi and Mak (1970). The percent recycling of glucose carbons is 25 - 30% in dogs and 10 - 15% in man. It appears, therefore, that recycling is highest in animals with the highest rates of glucose turnover (Katz et al., 1974b). Issekutz et al. (1972) showed that in dogs treated with glucocorticoids in which the Rt is about twice as high as in normal dogs, recycling was increased to 70 %. In normal rats, therefore, the estimated absolute rate of gluconeogenesis, as calculated from the turnover rate and recycling (0.235 x 7.43 mg/kg.min), equals 1.75 mg/kg.min. This calculation neglects the metabolic exchange in the hepatic oxalo-acetate pool and therefore underestimates gluconeogenesis by a factor of about 1.5 (Hetenyi, 1986). Taking this into account, the estimated rate of gluconeogenesis is 2.65 mg/kg.min.

In the rats treated with PZI, the Rt was seen to increase to 10.69 +/- 0.96 mg/kg.min. A very strong increase in the rate of production of glucose is seen in rat

in the face of even mild hyperinsulinemia. In these rats the rate of gluconeogenesis, uncorrected for metabolic exchange, was 2.14 mg/kg.min. In insulin induced hypoglycemic rats, the Rt equalled 11.58 +/- 1.07 mg/kg.min; the uncorrected rate of gluconeogenesis in this group was 2.04 mg/kg.min. In rats, therefore, there is a significant increase in the rate of appearance of glucose in response to mild or gross hyperinsulinemia, with or without hypoglycemia. This increased rate of glucose production appears to be primarily due to an increase in the rate of glycogenolysis or possibly due to an increased contribution to gluconeogenesis from amino acids. The contribution of gluconeogenesis in increasing the rate of production during hyperinsulinemia with or without hypoglycemia may be minimal in the rat, at least in short-term experiments. This contradicts Terrettaz et al. (1986), who have shown that insulin prevented glycogenolysis and spared glycogen in the fed rat, indicating that gluconeogenesis might be more important in providing glucose substrate.

In rats treated with an infusion of phlorizin (50 ug/kg.min), the Rt was seen not to increase significantly over normals. Hence, a decrease in the plasma glucose to 76 mg/dl was noticed in DSS. This may indicate that the rat has poor control over its plasma glucose around the set point, thereby suggesting that the rat can survive without any problems at a plama glucose concentration well below normal. The rat brain (and other obligate glucose

using tissues) may therefore be able to use other fuel sources as well as glucose under normal conditions, unlike dog or man.

After the injection of 35 mg/kg alloxan, no circulating IRI could be detected in the plasma. In these diabetic rats, a significant increase in the turnover rate (17% over basal) was observed. This confirms the earlier findings by Achou et al. (1974). A similar but larger increase has been shown to occur in the alloxan diabetic dogs (Hetenyi et al., 1980). Elevated plasma glucagon level is believed to be at least partially responsible for the increased rate of gluconeogenesis and consequently the higher turnover rate due to the lack of insulin mediated suppression of the alpha cells as occurs in normal animals (Unger et al., 1972; Bonner-Weir et al., 1982; Samols et al., 1983). In diabetic humans, elevated plasma glucagon levels have been reported (Foa, 1979).

In conclusion, therefore, non-compartmental analysis, indicated that the rat does increase its rate of glucose production in the face of an insulin challenge. The distribution space of glucose, however, does not seem to change, unlike previously reported studies that indicated increases in the volume of distribution of glucose under hyperinsulinemia in dogs (Hetenyi et al., 1961) and in man (Cobelli and Toffolo, 1984; Ferannini et al., 1985).

I.B.) Compartmental Analysis

Hetenyi et al. (1961) showed that insulin with or without hypoglycemia increased the apparent distribution space of glucose. They also showed that in depancreatized dogs the apparent glucose space did not increase during an insulin infusion.

In man, glucose has been shown to distribute itself into three compartments (Insel et al., 1975; Ferrannini et al., 1985). This three-compartmented mammillary system consisted of a central mixing compartment which represented the plasma, and two peripheral compartments, one of which represented insulin independent tissues (e.g., brain, splanchnic organs, erythrocytes and the renal medulla), and the other the insulin dependent tissues (e.g., skeletal muscle, etc.). They were able to show that under conditions of hyperinsulinemia, at normal plasma glucose levels, the volume of distribution of the insulin dependent compartment increases but that of the insulin independent compartment does not change.

In our experiments, for reasons discussed above, we were unable to fit our data to a sum of three exponential components. Thus, any 3-compartmented system collapsed into the general two compartment system (Refer to Appendix). This may indicate either that, in the rat, glucose is in fact distributed into two compartments, or more likely, we were unable to distinguish between the (small) first and

second compartments due to the fast turnover rate of glucose as compared to man or dog.

From Table IV it can be seen that upon using compartmental analysis we found that the size of the first compartment (V1) did not differ significantly among any of the experimental groups. In normal rats V1 = 164 +/- 8 ml/kg, in rats treated with PZI V1 = 154 +/- 8 ml/kg, in diabetic rats V1 = 156 +/- 16 ml/kg, in rats infused with phlorizin V1 = 153 +/- 12 ml/kg, in hyperinsulinemic hypoglycemic rats V1 = 174 +/- 15 ml/kg and in euglycmeic hyperinsulinemic rats V1 = 166 +/- 17 ml/kg. The volume of the first compartment in the rat is therefore very stable. It is not affected by hyperinsulinemia, nor is it affected by an increased clearance of glucose from the plasma.

The size of the second compartment in normal rats is of about the same volume as the first, V2 = 156 +/- 13 ml/kg. The rate of transfer of glucose between the two compartments is also nearly the same in normal rats. K21 equals 0.1393 +/- .02 min^{-1}, whereas the K12 equals 0.1284 +/- 0.012 min^{-1}. Using Norwich's model, where the rate constant of irreversible glucose elimination from the first compartment (K01) is set equal to that from the second (K02), it can be seen that K01 = K02 = 0.0183 +/- 0.00005 min^{-1}. Mild hyperinsulinemia as seen in the rats treated with PZI caused no change from normal in the size of V2 or in the rates of transfer of glucose between the two compartments. The rate constant of glucose elimination in

this mild hyperinsulinemic condition increased by 39% to .0254 +/- 0.0009 min^{-1}. In diabetes, a condition where there was no detectable insulin in the plasma, again the second compartment was of the same size as in normal rats. The rate constants for transfer of glucose between the two compartments however, were considerably above normal. K21 increased by 81% over baseline, and K12 increased by 56% over baseline. However, the K01 (=K02) decreased by 66% to 0.0063 +/- 0.0008 min^{-1}. Therefore, under a condition of relative hypoinsulinemia and hyperglycemia, the volumes of the compartments remained the same as in normal rats, but the rate of metabolism (elimination) of glucose from the compartments decreased considerably. In the hyperinsulinemic hypoglycemic rats, a marked increase in the volume of the second compartment (by 133% above baseline) was noticed. This was accompanied by an increase in the K01 (=K02) to 0.0483 +/- 0.004 min^{-1}, an increase of 164% above baseline. Furthermore, the fraction of glucose leaving the first compartment and entering the second compartment increased to 0.2095 +/- 0.032 min^{-1}, whereas, the fraction of the glucose in the second compartment entering the first compartment decreased to only 0.0471 +/- 0.003 min^{-1}. In the euglycemic hyperinsulinemic clamp, all the same changes that were observed in the hyperinsulinemic hypoglycemic rats occurred. This indicates that hyperinsulinemia, irrespective of plasma glucose concentration, causes an increased flux of glucose from the

first to the second compartment, causing the second compartment to act as a quasi-sink for glucose. The volume of the second compartment also increases irrespective of plasma glucose concentration under gross hyperinsulinemia, as does the fraction of glucose metabolized in the two compartments. (i.e., K01 (-K02) increases). To test whether this increase in the volume of the second compartment was in fact an insulin effect or if it was simply due to an increased flux of glucose from the first compartment, we increased the rate of elimination of glucose from the first compartment by an infusion of phlorizin. Similarly as in the hyperinsulinemic rats, K01 (-K02) was elevated to 109% above baseline, and K21 was elevated to 71% above baseline. In these rats the insulin concentration was below normal and the rats were mildly hypoglycemic. Therefore the elevations in the rate constants K01 (-K02) and K21 were not insulin-mediated effects; rather, they were caused by increased "leakage" of glucose from the first compartment caused by phlorizin. In these rats, no changes in the compartmental volumes were noted from normal. V2 remained stable at 219 +/- 20 ml/kg which was not significantly different from normal.

It should be noted that V2 is calculated assuming that C1 is equal to C2. As aforementioned, it is impossible to calculate one of these parameters independently of the other (DiStefano, 1982). Furthermore, as suggested by DiStefano (1982), the non-compartmental technique tends to

underestimate the true volume of distribution in all experimental groups, but this underestimation becomes statistically significant only in the gross hyperinsulinemic animals irrespective of the plasma glucose (Refer to Table IV).

In conclusion, therefore, the increases in the apparent distribution space caused by hyperinsulinemia that have been reported in the past to occur in man and in dog also occur in the rat. Mild hyperinsulinemia does not seem to affect compartmental volumes, although it increases glucose turnover and the rate constants K01 and K02. Gross hyperinsulinemia, with or without hypoglycemia, causes an even greater increase in the clearance of glucose from both the first and second compartments. The volume of distribution of the first compartment is remarkably stable under varying conditions of insulinemia. The second compartment was, however, seen to increase in volume markedly in response to gross hyperinsulinemia. Therefore, gradually raising plasma insulin levels above normal will first affect the rate constants, and only if the IRI is grossly increased will the volume of distribution of the second compartment increase.

This increase in the size of the second compartment due to insulin, and the relative robustness of the first compartment irrespective of IRI, leads us to believe that the first compartment may in fact be primarily a mixing

compartment, whereas the second compartment may consist of tissues that are insulin sensitive (e.g., skeletal muscle). There is some metabolism of glucose in the first compartment; therefore, we postulate that the first compartment may consist of insulin independent tissues (e.g., brain, renal medulla, etc.) and the mixing compartment as proposed by Ferrannini et al. (1985) for man. It is possible, therefore, that if we were able to sample early enough after the injection of tracer, that we would be able to separate the mixing and the insulin sensitive fractions of the first compartment as Ferrannini et al. (1985) did in man.

The increase in the size of the second "insulin sensitive" compartment under conditions of hyperinsulinemia irrespective of plasma glucose concentration may be explained by three possible explanations. a) The physical space of glucose has actually increased. This is unlikely. However, it is possible that glucose becomes deposited as glycogen in the insulin dependent tissues thereby increasing the virtual volume of glucose without a proportional actual increase in the volume. b) More of the intracellular space becomes accessible to glucose, and part of the intracellular water exchanges with extracellular glucose (Hetenyi et al., 1961). c) There is increased cycling between free glucose and glycogen, the latter of which can act as a sink for glucose.

I.C.) K01 Clamp

Compartmental analysis led us to conclude that the first compartment, because of its constancy, is primarily a mixing compartment that also contains the glucose uptake by insulin insensitive tissues, and into which glucose is released. The second compartment, on the other hand, represents glucose uptake by insulin sensitive tissues. The size of the second compartment increases during the infusion of insulin.

In order to better characterize the glucose distribution system, it was attempted to distinguish between the two compartments, insulin insensitive and insulin sensitive, with respect to their ability to metabolize glucose under varying insulin concentrations. The first compartment may in fact be considered to be of the same volume under all experimental conditions. That is, the first compartment will metabolize glucose with a steady rate constant, irrespective of plasma IRI. This is also supported by the finding that the volume of the first compartment is independent of plasma IRI and/or glucose. We therefore assumed that in all cases the first compartment follows the same kinetics as in the normal rat. The K01 was therefore clamped at 0.0183 +/- 0.00005 min^{-1}. We then calculated the K12*, K21* and K02* with K01 clamped. This new addition to the routine model will be referred to as the "K01-Clamp" model. Using the K01 -Clamp, we found that the

intercompartmental rate constants of glucose transfer (i.e. K12* and K21*) did not differ significantly from their respective K12 and K21 as calculated using the routine Norwich (1977) model. The K01-Clamp model, proved however, to provide some very interesting values for K02*. In, mild hyperinsulinemia, we would expect the K02* to increase above K01. This new model showed exactly that: K02* increased from 0.0254 +/- 0.009 min^{-1} using the routine model to 0.0315 +/- 0.001 min^{-1} when K01 was clamped. In gross hyperinsulinemia, we would expect an even greater increase in K02*. Again, the model showed an increase in the K02*. The K02* in the hyperinsulinemic hypoglycemic rats increased from 0.0483 +/- 0.004 min^{-1} to 0.0528 +/- 0.009 min^{-1}. Similarly, the K02* in the euglycemic-hyperinsulinemic clamped rats increased from 0.0749 +/- 0.0005 min^{-1} to 0.0844 +/- 0.01 min^{-1}. In the rats infused with phlorizin, we would expect a decrease in the K02, as IRI was lower in these rats than in normals. The new model, however, predicted an increase in K02* above K01. K02* increased from 0.0382 +/- 0.002 min^{-1} to 0.0474 +/- 0.009 min^{-1}. In diabetes would predict that the ability of the second to metabolize glucose would decrease. The model predicted a decrease, with the calculated K02 with the clamped K01 dropping from 0.0063 +/- 0.0008 min^{-1} to <u>-0.0004 +/- 0.0009 min^{-1}</u>. A negative rate constant is impossible, and suggests that the calculated K02* may infact equal zero. Its value indicates that in diabetes, in the rat, the insulin

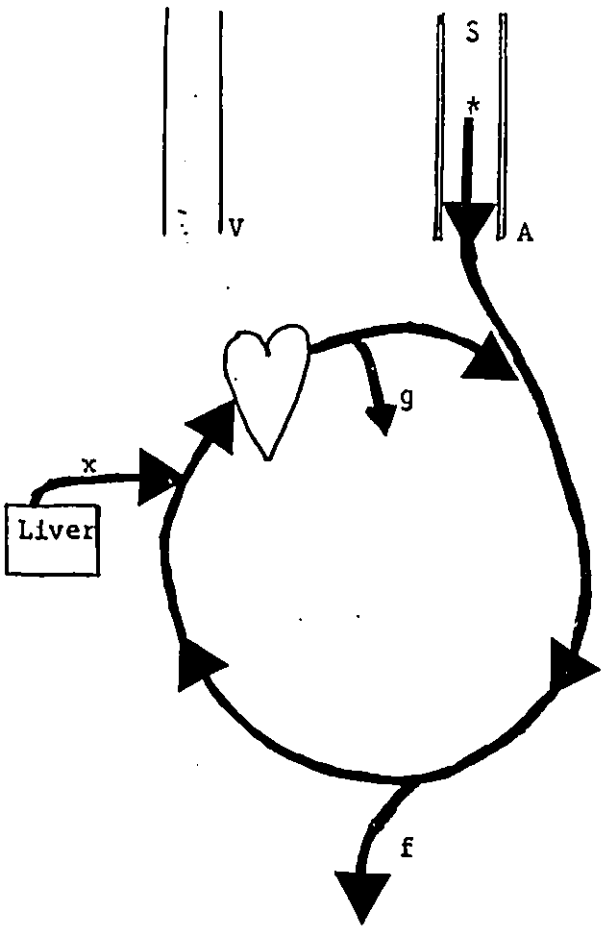
sensitive tissues do use glucose to a very limited degree. The KO1-Clamp model therfore, predicted a plausible change in the KO2 in all cases except possible for the phlorizin-treated rats. Evidently, therefore, a more sophisticated approach to clamp KO1 may have to be sought after.

I.D.) Normal Rats: A/A vs. A/V Design

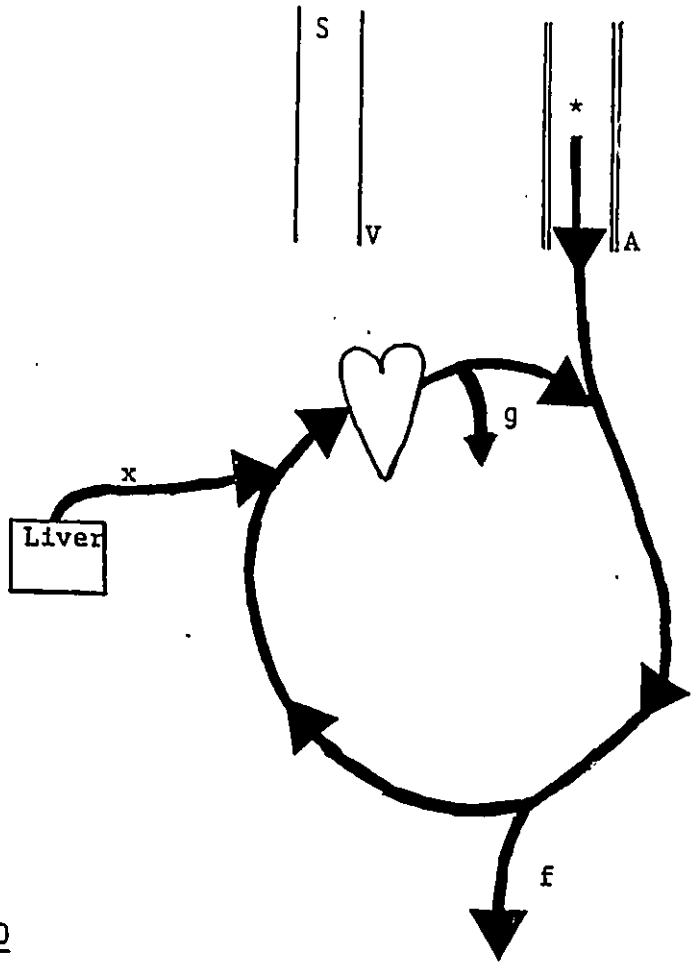
In normal rats Okajima et al. (1981) found that the V/A (venous injection of tracer followed by arterial sampling) calculated the turnover rate of lactate is 47% smaller than the one calculated after arterial injection of tracer followed by venous sampling (A/V).

FIGURE 14: Schematic representation of possible explanation for the difference in calculated turnover rate (Rt) between A/A (Arterial injection of tracer followed by arterial sampling) and A/V (Arterial injection of tracer followed by venous sampling) designs.

A/A



A/V



LEGEND

x = input of unlabelled glucose from the liver

* = injection of tracer

f = fractional extraction of glucose by brain and splanchnic bed tissues

g = fractional extraction of glucose by lungs and heart muscle

S = sampling site

A/A

Both f and g remove tracer glucose before 1st sampling.

$Rt = (1 + f + g) * x$

A/V

f removes tracer glucose before 1st sampling.

$Rt = (1 + f) * x$

Therefore, Rt (A/A) is greater than Rt (A/V)

This was to be expected as lactate enters the system at multiple points. Hence, if sampling is to be from a point that can be taken to be at the outflow of the system, then the A/V design puts the tracer and the tracee into the same capillary bed and allows for the collection of mixed venous blood at the outflow. This would account for the discrepancy in the calculation of the turnover for lactate (Okajima et al., 1981). The same group showed that for the Rt for glucose, a compound that enters the circulation at a single point into the vena cava, there was no significant difference between the A/V and V/A designs. We conducted a series of experiments in normal rats in the p.a. state, using the A/A design and A/V design, and found that the Rt in the A/A group was 7% greater than the Rt in the A/V group. An explanation for this difference may be seen in Figure 14. Basically, the only difference between the two techniques, is that in the A/A design the tracer is collected after it has passed through both the systemic and pulmonary circulations; whereas in the A/V design blood from the latter is not included at the point of sampling (outflow). The clearance of tracer from the system for any given time (t) will therefore be greater in the A/A case than in the A/V case due to the glucose extraction abilities of the heart and lungs. The heart has been shown to utilize about 4% of the circulating glucose (and presumably tracer) in the p.a. condition (Neely et al., 1974), and the lungs less than 1% (Ferrannini et al., 1985). This 5% difference

in the total glucose utilization may therefore be enough to account for the differences we have detected in our two designs. It is concluded, therefore, that for an actual estimate of whole body turnover of glucose in the p.a. state in the rat, the use of either the A/A or the V/V design is preferable since the A/V design does not give an accurate estimate of whole body glucose utilization.

I.E.) Euglycemic Clamp: Incomplete Suppression of hepatic glucose production

The euglycemic clamp technique was developed as a means for quantifying the *in vivo* insulin sensitivity in man (Andres et al., 1966). Kraegen et al. (1983) showed that essentially the same techniques as used in man were applicable to the rat. The technique basically is a process whereby a given level of plasma glucose is maintained during the infusion of insulin by the simultaneous infusion of glucose. If the desired level of glucose is near that in the normal fasted animal, the "clamp" is said to be euglycemic. The calculated rate of appearance of glucose (equal to the calculated turnover rate) then would equal the glucose infusion rate if the hepatic glucose output equals zero and gut glucose absorption is assumed to equal zero in the p.a. state (Proietto et al., 1986). Rossetti et al. (1987) found a 95 - 97% suppression of Ra. The good agreement between the calculated Rt and the infusion rate of glucose would indicate that endogenous glucose production

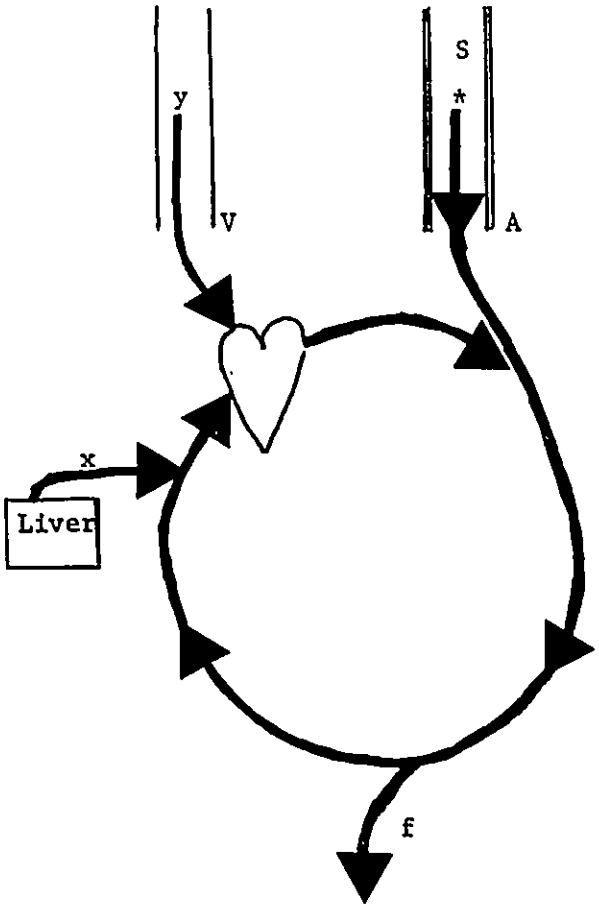
was entirely suppressed. Nevertheless, we found that 5% of the Rt was from gluconeogenesis, as estimated by the difference between the Rt as calculated by 14-C and 3-H glucose, seem to contradict this interpretation. It is possible, therefore, that suppression was only 95% effective and the 5% difference was too small to be detected by the tracer method. This indeed is quite possible in the light of the already quoted results by Radziuk et al. (1978), that the errors in the tracer methods ranges between 7.5 and 10%. However, Insel et al. (1975) have found that liver glucose production was suppressed by only 70% during a euglycemic clamp in p.a. men. Cobelli et al. (1987) have found an incomplete suppression of the production of glucose (62 to 83% suppression) by the liver during a euglycemic clamp in women in the p.a. state. Finegood et al. (1987) have also found that in dogs in the p.a. state, the suppression of Ra ranged between 60 and 100%.

I.F.) The Euglycemic Clamp: AVA, VAV, AAV, VVA Designs

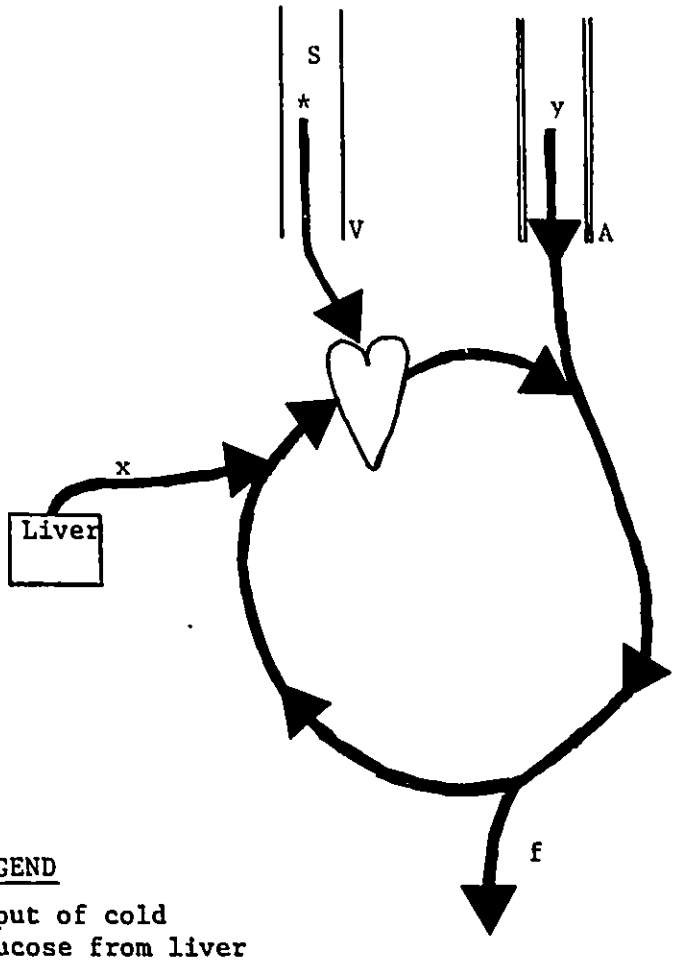
In hyperinsulinemic rats the turnover rate of glucose is elevated markedly. We therefore wanted to test whether varying the sites of tracer injection, tracee infusion and sampling would make a difference in the calculated turnover rate. From Table II it can be seen that in the VAV case the estimation of the infusion by the calculated turnover rate is accurate, whereas the AVA design overestimated the infusion rate. A possible explanation for this discrepancy may be found in Figure 15.

FIGURE 15: Schematic representation of a possible explanation for the difference in the calculated turnover rate (Rt) between the AVA (arterial injection of tracer, venous infusion of tracee, and arterial sampling) and the VAV (venous injection of tracer, arterial infusion of tracee, and venous sampling) designs in normal rats in the p.a. state.

AVA



VAV



LEGEND

x = input of cold glucose from liver
y = infusion of cold glucose
* = infusion of tracer
f = fractional extraction by tissues
S = sampling site

AVA

f removes tracer glucose (*) first. This is replaced by x (unlabelled glucose) from the liver.

R_T (=Ra) = $(1+f)\cdot(x+y)$

VAV

f removes tracee glucose (y) first. This is replaced by x (unlabelled glucose) from the liver.

R_T (=Ra) = $x + (1-f)\cdot y$

$\therefore R_T\text{-AVA} > R_T\text{-VAV}$

The AVA case possibly overestimates the actual infusion rate because, upon injection of tracer into the artery and infusion of tracee into the vein, the splanchnic bed (a major site for glucose extraction) will see tracer glucose first, before it has mixed with tracee glucose. Muratoglu et al. (1986) have shown that the first pass hepatic uptake and utilization of glucose in the rat is over 13%. If in fact there is incomplete suppression of the liver Ra the relative SA of the tracer glucose will be decreased, and an elevated Rt will be obtained. In the VAV case, the splanchnic bed sees primarily tracee glucose first. This is replaced by the liver Ra, and therefore an accurate estimate of the infusion rate by the calculated turnover rate will result.

If equivalent tracer supply conditions are met, then the calculation of the infusion rate of glucose is fairly accurate in both AAV and VVA situations. This technique avoids the problems associated with the AVA or VAV designs. We suggest, therefore, that when the euglycemic clamp technique is used, to obtain an accurate estimate of the turnover rate, equivalent tracer supply conditions must be satisfied.

II.) EXPERIMENTS OUT OF DYNAMIC STEADY STATE (ODSS)

In the ODSS situation, we suggest that due to the large changes in the apparent volume of distribution and the rate

constants between normal and hyperinsulinemic rats, that the one compartment appproximation of Steele (1959) (with the pV equal to V1 = 165 ml/kg) be used. This is suggested because the increase in the size in the second compartment due to the gross hyperinsulinemia could not be attributed to an actual physical increase in the glucose space. In other words, some of the glucose may in fact have been stored as intracellular glycogen, thereby giving an overestimation of the increase in V2. The use of time variant K's as suggested by Cobelli et al., (1987) is another alternative, left unexplained in this study.

III:) GLUCOREGULATION IN THE RAT

As shown on Figure 12, rats regulate effectively against overt insulin induced hypoglycemia. The magnitude of the increase in Ra is at least as high as in dogs (Gauthier et al., 1982).

On the contrary, non-hypoglycemic glucoregulation was much less effective (Figure 13) than observed in dogs (Gauthier et al., 1980), since the elevation of Ra in response to the infusion of phlorizin was small and transient. As a result, plasma glucose was decreased by 47% from the basal p.a. level. The cause of this difference between rats and dogs has not been investigated. However, glucoregulation near the set point seems in general to be

(1987) found that fed rats develop hyperglycemia during exercise. This was not seen in dogs (Issekutz et al., 1979) or men (Wahren et al., 1971; Vranic et al., 1979; Issekutz et al., 1979).

CHAPTER VI

CONCLUSIONS:

1) Gradually raising plasma insulin levels above normal (irrespective of plasma glucose) will first affect the rate constants K01 and K02, and only if it is grossly increased will the K12 and the volume of the second compartment increase and K21 decrease.
2) The size of the first compartment (V1 = 165 ml/kg) is not affected by either plasma IRI or glucose level. Its size approximates that of the extracellular volume and the insulin insensitive tissues.
3) With tracer injected into an artery and blood sampled from the artery (A/A) in normal rats, the calculated steady state turnover rate was 7% higher than with the A/V mode. In euglycemic hyperinsulinemic rats, the arterial injection of tracer, the venous infusion of tracee and the arterial sampling of plasma, resulted in a 11% overestimate of the glucose infusion rate. Under equivalent tracer conditions A/V and V/A techniques gave the same calculated results.
4) In experiments out of steady state it is suggested to use V1 instead of pV as the volume in the single compartment approximation of the glucose system.
5) Rats glucoregulate by increasing their rate of glucose production (Ra) in response to overt hypoglycemia.
6) Non-hypoglycemic glucoregulation does not operate efficiently in the rat.

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APPENDIX

The triple exponential equation as shown below was used to calculate the rate coefficients and volumes of the different three-compartment models. As can be seen, the first (rapid) exponential does not differ significantly from the second exponential ($p > 0.05$). Therefore, we may consider that both of these exponentials are in fact indistinguishable. Therefore, all three-compartmental models based upon this equation collapsed into a two-compartmental system. Twelve plasma samples were analyzed (12 point schedule). This would indicate that: a) the rat glucose system is in fact only a two-compartmental system, or b) the turnover rate of glucose is much too quick in the rat to be able to withdraw samples frequently enough after the injection of tracer in order to find the first (rapid) exponential necessary to characterize the kinetics of the first compartment. Note that in man and in dog, the three-compartmental representation of glucose has been shown to be a plausible one (Foster et al., 1980; Insel et al., 1975; Cobelli and Toffolo, 1984, Ferrannini et al., 1985).

$$C^*(t) = B1\, e^{-b1(t)} + B2\, e^{-b2(t)} + B3\, e^{-b3(t)}$$

where: B1 = 647187 +/- 582602; b1 = 0.35763 +/- 0.18019

B2 = 420756 +/- 94215; b2 = 0.17779 +/- 0.08526

B3 = 417522 +/- 35220; b3 = 0.01703 +/- 0.00288