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LA THÈSE A ÉTÉ
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NOUS L'AVONS REÇUE

A Gilayne, mon amour,
pour sa patience et
sa foi en moi...

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LIST OF FIGURES

FIGURES	PAGES
1. The effect of an infusion of phlorizin ($50 \mu\text{g.kg}^{-1} \text{min}^{-1}$) followed by that of phlorizin plus somatostatin ($0.35 \mu\text{g.kg}^{-1} \text{min}^{-1}$) on the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on glucose appearance (Ra) and disappearance (Rd).	15
2. Regulation of glycogen metabolism in the liver.	20
3. Entry of precursors into the pathway of gluconeogenesis.	24
4. Schematic representation of the metabolic exchange of C-atoms in the hepatic oxaloacetate (OAA) pool.	27
5. Model used in the calculation of the fluxes of carbon-atoms across and between the plasma alanine and glucose pools.	53
6. Model used in the calculation of the recirculation of carbon-atoms within the glucose molecule and the flux of glucose from the hepatic glycogen pool to plasma.	55
7. Schematic representation of the double-compartmented model of the glucose system.	59
8. Model used in the calculation of the fluxes of carbon atoms across and between the plasma glycerol and glucose pools.	60
9. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1} \text{min}^{-1}$ in dogs in the p.a. state, on plasma concentration of immunoreactive glucagon (IRG), plasma glucose concentration, rate of appearance (Ra) and clearance rate (CR) of glucose, rate of appearance (Ra) of alanine,	65

rate of formation of glucose from alanine, fraction of circulating glucose arising from alanine and the fraction of utilized alanine-C transferred to glucose.

10. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the fasted state on parameters listed in Fig. 9. 68
11. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state on plasma concentration of immunoreactive glucagon (IRG), plasma glucose concentration, rate of appearance (Ra) and clearance rate (CR) of glucose, rate of glycogenolysis and rate of recirculation of ^{14}C -atoms into glucose. 71
12. Effect of infusing insulin at $7 \text{mU.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state on plasma concentration of immunoreactive glucagon (IRG), plasma glucose concentration, rate of appearance (Ra) and metabolic clearance rate of glucose (MCR), rate of glycogenolysis and rate of recirculation of ^{14}C -atoms into glucose. 75
13. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state, on plasma glucose concentration, rate of appearance (Ra) of glucose, plasma glycerol concentration and rate of appearance (Ra) of glycerol. 78
14. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state, on the rate of formation of glucose from glycerol, the fraction of circulating glucose arising from glycerol, the fraction of utilized glycerol-C transferred to glucose, the specific activity of lactate and the plasma lactate concentration. 80
15. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state, on plasma concentration of glucagon, adrenocorticotropin (ACTH), cortisol and epinephrine. 82

16.	Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the fasted state on parameters listed on Fig. 13.	87
17.	Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the fasted state on parameters listed on Fig. 14.	89
18.	Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the fasted state on parameters listed on Fig. 15.	91
19.	Anthony's Cannula: A. disassembled B. assembled	125
20.	Determination of the concentration of glycerol.	138

LIST OF TABLES

TABLES	PAGE
1. The turnover rate of alanine and the contribution of carbon atoms from plasma alanine to circulating glucose. Mean values are shown.	28
2. The turnover rate of glycerol and the rate of carbon transfer between plasma glucose and glycerol in the postabsorptive state at rest.	31
3. The rate of appearance of glucose in dogs calculated before, during and after the infusion of phlorizin between the one and two-compartmental model.	72
4. The ratio of SA(glucose)/SA(lactate) in the plasma of dogs in the postabsorptive (P.A.) state and after a 4 day fast.	92
5. The effect of the infusion of phlorizin on the plasma alanine concentration ($\mu\text{mole/L}$) in conscious dogs in the postabsorptive and fasted (96 hrs) states.	93
5.1 ^{14}C -glycerol activity (G^*), glycerol concentration (G) and specific activity (SA) in the cuvette for untreated and purified samples calculated by methods a and 2 (see text).	139
6. to 35 Statistical analysis of results (First Series)	143 to 172
36 to 41 Statistical analysis of results (Second Series)	173 to 178
42 to 75 Statistical analysis of results (Third Series)	179 to 212
76 to 83 Quality control	214 to 221

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	
LIST OF FIGURES	III
LIST OF TABLES	VI
TABLE OF CONTENTS	VII
I - SUMMARY	1
II - INTRODUCTION	6
III - REVIEW OF THE LITERATURE	11
Nonhypoglycemic vs Hypoglycemic Glucoregulation	11
a. Hypoglycemic Glucoregulation	11
b. Nonhypoglycemic Glucoregulation	12
Glycogenolysis	19
Gluconeogenesis	23
a. The Synthesis of Glucose from Alanine	25
b. The Synthesis of Glucose Measured by the Recirculation of ¹⁴ C-atoms Into Plasma Glucose	29
c. The Synthesis of Glucose from Glycerol	29
d. Gluconeogenesis in the Postabsorptive and Fasted State.	32
IV - MATERIALS AND METHODS	
Animals	34
Procedures	34

Experimental Design	36
Blood Samples	38
Chemicals	38
Determination of the Concentration of Metabolites and Hormones in Plasma	39
a. Assays Used in All Series of Experiments	39
a.1. Determination of Glucose	39
a.2. Determination of ^3H -3-glucose and ^3H -6-glucose	40
a.3 Determination of Glucagon	41
b. Assays used in Individual Series of Experiments	41
b.1. First Series	41
b.1.1. Determination of Alanine in Plasma	42
b.1.2. Determination of ^{14}C -U-alanine in Plasma	42
b.1.3. Determination of ^{14}C -glucose in Plasma	42
b.2. Second Series	43
Determination of the Concentra- tion of ^{14}C -atoms Reincorporated into Plasma Glucose Molecule	43
b.3. Third Series	43
b.3.1. Determination of ^{14}C -glucose in Plasma	43
b.3.2. Determination of Lactate in Plasma	44
b.3.3. Determination of the Concentration of ^{14}C - Lactate in Plasma	44

b.3.4. Determination of Glycerol in Plasma	45
b.3.5. Determination of ^{14}C -U-Glycerol in Plasma	46
b.3.6. Determination of Epinephrine in Plasma	47
b.3.7. Determination of Cortisol in Plasma	47
b.3.8. Determination of ACTH in Plasma	48
Calculations	49
a. General	49
a.1. Rates of Appearance and Disappearance of Glucose	49
a.2. Rates of Appearance and Disappearance of Alanine	50
a.3. Rates of Appearance and Disappearance of Glycerol	51
a.4. Statistical Analysis	51
b. Calculations Used in Individual Series of Experiments	51
b.1. First Series	51
Gluconeogenic Rate, Fraction of Circulating Glucose Coming from Gluconeogenesis and Fraction of the Utilized Precursor Going into Glucose	
b.2. Second Series	54
b.2.1. Gluconeogenic Rate (recirculation of ^{14}C -atoms into Glucose)	54
b.2.2. Glycogenolytic Rate	57

	b.2.2.1. One-Compartment Model	57
	b.2.2.2. Two-Compartments Model	57
	b.3. Third Series	
	b.3.1. Gluconeogenic Rate, Fraction of Circulating Glucose Coming From Gluconeogenesis and Fraction of the Utilized Precursor Going into Glucose	58
	b.3.2. The Fraction of Circulating Glucose Coming From Lactate	62
	b.3.3. Statistical Analysis	62
V	- RESULTS	63
	First Series:Gluconeogenesis from Alanine in Nonhypoglycemic Glucoregulation	63
	Second Series:Glycogenolysis in Nonhypoglycemic and Hypoglycemic Glucoregulation	69
	Third Series:The Role of Glycerol and Epinephrine,ACTH and Cortisol in Nonhypoglycemic Glucoregulation	73
VI	- DISCUSSION	94
	First and Second Series:Gluconeogenesis and Glycogenolysis in Nonhypoglycemic and Hypoglycemic Glucoregulation	94
	a. The sources of Plasma Glucose in the Postabsorptive and Fasted States	94
	b. The Response to an Increased Removal of Glucose from the Circulation	96

c. The Response to Insulin Induced Hypoglycemia	101
Third Series: The Role of Glycerol, Epinephrine, ACTH and Cortisol in Nonhypoglycemic Glucoregulation	104
a. Glycerol and Glucose Metabolism in the Postabsorptive State and After Four Days' Fasting	104
b. The Conversion of Glycerol to Glucose and Lactate	106
c. Glycerol as a Source of Glucose Released During the Infusion of Phlorizin	107
d. The Release of Epinephrine, ACTH and Cortisol	110
VII - BIBLIOGRAPHY	114
VIII- APPENDIX	124
I. Construction and Implantation of a Permanent Cannula	125
a. Cannula Construction	125
b. Cannulation and Implantation	126
II. Determination of the Concentration and Specific Activity of Plasma Glycerol in a single assay	128
III. Calculation of V_1 , K_{12} and K_{21} (2-compartmental model)	140
IV. Statistical Analysis of Results	142
First Series	143
Postabsorptive state	143
Fasted State	152
Postabsorptive vs Fasted States	159

Second Series . . .	173
Third Series	179
Postabsorptive State	179
Fasted State	193
Postabsorptive vs Fasted States	207
V. Quality Control	213
Plasma Glucose	214
Plasma alanine	215
Plasma Glycerol	216
Plasma Lactate	217
Plasma Glucagon	218
Plasma Epinephrine	219
Plasma ACTH	220
Plasma Cortisol	221

I- SUMMARY

When the rate of removal of glucose from plasma is increased by the infusion of 50 $\mu\text{g/kg.min}$ phlorizin (PHL) the rate of hepatic glucose release (R_a) increases, so that the concentration of glucose in plasma falls only marginally. The response is mediated by glucagon (Gauthier et al., 1980). The purpose of this study was to establish to what extent glycogenolysis and gluconeogenesis contribute to the increment in R_a mediated by glucagon released in response to a physiological stimulus: the increased rate of removal of glucose from the plasma.

Three series of experiments were carried out on unanaesthetized dogs, unselected for sex and trained to stand quietly in a Pavlov stand. In the first series, dogs in the postabsorptive (p.a.) state were infused with trace amounts of ^3H -3-glucose and ^{14}C -U-alanine. R_a and the clearance of glucose from the plasma (CR) were calculated together with the irreversible rate of removal (R_i) of alanine and the rate of glucose formation from plasma alanine before, during and after the infusion of PHL. When R_a (glucose) was increased during the infusion of PHL, the synthesis of glucose from alanine was also increased. This was achieved by an increase in the fraction

of utilized alanine which was converted to glucose, since Ri (alanine) remained unchanged. The experiment was repeated after 4 days of fasting, when the rate of gluconeogenesis was twice that in the p.a. state; the Ra (glucose) still increased during the infusion of PHL, but to a lesser degree and somewhat more slowly than in the p.a. state. The concentration of glucose in the plasma fell by about 30mg/dl but the Ri (alanine) remained unchanged.

In the second series of experiments hepatic glycogen stores were pre-labelled with ^3H -6-glucose three days before ^{14}C -1-glucose was infused as the tracer. The appearance of ^3H -atoms in plasma glucose was taken as a measure of the rate of glycogenolysis. During the infusion of PHL the rate of glycogenolysis was doubled as was the recirculation of ^{14}C -atoms from the first to the sixth position in plasma glucose, indicating an increase in the activity of the combined Cori and alanine-glucose cycles.

Considered together, the results of the first two series of experiments suggest that: (1) glucagon released in response to an increased removal of glucose from plasma increases the rate of glucose production (nonhypoglycemic glucoregulation) by stimulating both gluconeogenesis from alanine and breakdown of hepatic glycogen; (2) In fasted dogs in which the basal rate of gluconeogenesis was twice as high as that in the p.a. state, PHL infusion caused a further increase.

The increase was achieved by a larger fraction of utilized alanine being converted to plasma glucose.

In the third series of experiments, ^{14}C -U-glycerol and ^3H -3-glucose were infused in trace amounts to dogs in the p.a. state and after 4 days of fasting. Plasma concentrations of glucagon, epinephrine, cortisol and ACTH, and the concentration and specific activity (SA) of plasma lactate, were also monitored. In dogs in the p.a. state during the infusion of PHL: (a) An increase in the rate of glucose production again nearly compensated for the urinary loss of glucose; (b) The rate of glycerol release into the circulation was increased, but the same fraction of utilized glycerol was converted to glucose as before the infusion, so that the fraction of circulating glucose arising from glycerol increased; (c) While the concentration of glucagon in the plasma was doubled, that of the other hormones did not change; (d) Nor was there any change in the level and SA of plasma lactate. In dogs fasted for 4 days: (a) The rate of glucose production increased; nevertheless, plasma glucose dropped by 25 mg/dl; (b) Although the rate of glycerol release was not significantly raised, a larger fraction of utilized glycerol was converted to glucose indicating that a large part of C-atoms from glycerol reached glucose by conversion to lactate and the Cori cycle; (c) The plasma concentrations of glucagon, ACTH and

cortisol, but not of epinephrine, increased; and (d) so did the SA but not the level of plasma lactate. The results of the third series of experiments indicate that during the infusion of PHL, (1) glycerol contributes only to a minor extent to the increment of hepatic glucose production; (2) Extra epinephrine is not released together with glucagon in either the p.a. or fasted state; (3) ACTH and cortisol are released only in fasted dogs in response to the relatively large fall in plasma glucose which occurs in these dogs. The release of ACTH is closely correlated with an increase in the SA of plasma lactate; (4) In fasted dogs glycerol appears to be a substrate more of oxidation than gluconeogenesis.

In summary, the results of this study have shown that phlorizin-induced nonhypoglycemic glucoregulation is mediated by glucagon and achieved through an increase in glycogenolysis and gluconeogenesis. Second, the increase in gluconeogenesis from alanine is achieved by a larger fraction of utilized alanine converted to glucose. Finally, it appears that glycerol contributes only to a minor degree in PHL-induced gluconeogenesis. It functions more as a substrate for oxidation than gluconeogenesis.

II - INTRODUCTION

During phlorizin induced glycosuria, when about 1 mg/kg.min glucose is spilled, the level of glucose in the plasma falls only by 10-18 mg/dl. A further decline in plasma glucose level is prevented by a compensatory increase in the rate of hepatic glucose release. This response has been shown to be mediated by glucagon, the effect of which might be augmented by a simultaneous marginal decrease in the concentration of plasma insulin (Gauthier et al., 1980).

The increase in hepatic glucose production in response to phlorizin glycosuria represents a situation in which a single hormone (glucagon) is released by a physiological stimulus (the small decrease in the level of plasma glucose due to an increased rate of removal of glucose from the circulation), in a regulatory response which achieves its purpose (the level of plasma glucose remains near to its set point). This regulation has been recently referred to as "nonhypoglycemic glucoregulation" by Cryer et al. (1981). One would expect that a homeostatic regulation, such as the maintenance of normoglycemia, will not depend on the availability of a single enzymatic mechanism. Glucagon indeed has well documented effects on both glycogenolysis (Exton et al., 1970) and gluconeogenesis (Chiasson et al., 1974) when infused i.v. at a high enough

rate (Chiasson et al., 1975). The general purpose of this study was to test the hypothesis that increased hepatic glucose production in nonhypoglycemic glucoregulation is mediated by glucagon by multiple routes. Three series of experiments were performed.

In the first series, the rate of hepatic glucose production and the contribution of gluconeogenesis from alanine to the extra glucose release during an infusion of phlorizin were determined in dogs in the p.a. and the fasted state. Four specific questions were addressed:

- (1) Does hepatic glucose production increase during the infusion of phlorizin to the same extent and does it follow the same temporal pattern in glycogen-depleted and p.a. dogs ?
- (2) If so, to what extent does gluconeogenesis from alanine contribute to the extra glucose released?
- (3) If gluconeogenesis from alanine occurs at a higher rate, does this result from an increased turnover of alanine or because of an increase in the fraction of alanine turnover converted to glucose, as is the case with supraphysiological doses of glucagon (Chiasson et al., 1975) ?
- (4) Finally, is the contribution of alanine to plasma glucose further increased in fasted dogs during nonhypoglycemic glucoregulation?

Glycogen has been shown to be an important source of glucose in dogs in the p.a. state (Bishop et al., 1965).

As already mentioned, glucagon stimulates glycogenolysis (Exton et al., 1970). The purpose of the second series of experiments was to determine the relative contributions and time patterns of glycogenolysis and gluconeogenesis in nonhypoglycemic glucoregulation, using the technique developed by Radziuk and Vranic (1977) for labelling of liver glycogen. In these experiments, the contribution by gluconeogenesis was measured by the re-incorporation of ^{14}C -atoms into the glucose molecule (Forbath et al., 1969).

In order to ascertain whether glycerol, another recognized substrate of gluconeogenesis (Hall et al., 1976), also contributes to the extra glucose released during the infusion of phlorizin, a third series of experiments was undertaken. This problem was of particular interest, since glycerol is released into the plasma as a product of lipolysis, a process not under the control of glucagon, but regulated primarily by catecholamines and to a lesser extent by a direct effect of ACTH (Engel, 1961) on fat cells. Furthermore, should ACTH be mobilized by such a small decrease in the concentration of glucose in the plasma, the resulting increase in the secretion of adrenal glucocorticoids may be expected to increase the sensitivity of the lipolytic process to catecholamines (Issekutz and Allen, 1972).

The turnover of glycerol and its contribution to the formation of plasma glucose was calculated during the infusion of phlorizin in dogs in the p.a. and in the fasted state. The concentration of glucagon, epinephrine, ACTH and cortisol in the plasma were also pre-determined intervals to establish which of these hormones may mediate the production of extra glucose.

Following recent in vivo evidence for a rapid utilization of small quantities of glycerol in the adipose tissue, without the need for its previous conversion to glucose by the liver (Carmaniu et al.; 1980), the specific activity of lactate was also measured as an index of increased transport of ^{14}C -atoms from glycerol towards the Krebs cycle. The specific questions addressed were: (1) Are all glycerol carbon atoms of utilized glycerol going "upstream" towards glucose or can glycerol be channelled "downstream" towards the Krebs cycle, thus simultaneously providing energy for the cell and a precursor for glucose in the form of lactate? (2) Is the "downstream" pathway sensitive to any metabolic change taking part in nonhypoglycemic glucoregulation during the infusion of phlorizin and/or to differences in nutritional status? (3) Is there a potential hormonal candidate mediating glucoregulation through this mechanism?

In summary, glucagon has been shown to mediate the nonhypoglycemic glucoregulation in dogs in the p.a. state (Gauthier et al., 1980) by stimulating both glycogenolysis (Exton et al., 1970) and gluconeogenesis (Chiasson et al., 1975). Alanine and glycerol are recognized precursor of gluconeogenesis (Hetenyi, 1981). The purpose of this study was to determine the relative contribution and temporal pattern of activation of glycogenolysis and gluconeogenesis (from alanine and glycerol) during phlorizin-induced nonhypoglycemic glucoregulation in dogs in the p.a. and fasted state. The concentration of glucagon, ACTH, cortisol and epinephrine in the plasma were also determined under both nutritional states in order to clarify their role as hormonal signals of the nonhypoglycemic glucoregulation.

III-REVIEW OF THE LITERATURE

Nonhypoglycemic vs Hypoglycemic Glucoregulation

a. Hypoglycemic Glucoregulation

The maintenance of the plasma glucose concentration above a critical level is crucial to survival, since glucose is an essential fuel for the central nervous system. The complex system by which the body maintains this concentration is referred to as "glucoregulation". Several hormones as well as the nervous system take part in glucoregulation.

Insulin is well-known as a potent hypoglycemic agent. The rise in plasma glucose following hypoglycemia produced by the i.v. injection or infusion of insulin has been the model most extensively used to dissect the mechanisms of acute glucose counter^regulation. This type of regulation

is in response to overt hypoglycemia, the so-called "hypoglycemic glucoregulation". Insulin-induced hypoglycemia calls for an immediate increase of hepatic glucose production mediated at least in part by glucagon, since the infusion of somatostatin delayed the return of plasma glucose concentration to normal in man (Rizza et al., 1979) and to a lesser extent in dogs (Gauthier et al., 1980). However, the fact that the restitution of normoglycaemia fails to occur only when the release of both glucagon and epinephrine are prevented

(Rizza et al., 1979) shows that the hypoglycemic counter-regulation is achieved mainly via catecholamines.

Indeed catecholamines have been shown to be released in large amounts during deep (De Fronzo et al., 1977; Goldfien et al., 1958; Wallace and Harlan, 1965), but not marginal (Christensen et al., 1975; De Fronzo et al., 1977) hypoglycemia. It should also be noted that cortisol and growth hormone are also released during insulin-induced overt hypoglycemia (Garber et al., 1976) but their acute release is not critical in the process of re-establishing normoglycemia (Cryer, 1981).

Even though these findings are important to an understanding of the physiologic mechanisms of activation of the counter-regulatory system, their relevance to nonhypoglycemic glucose regulation remained questionable, until recently (Gauthier et al., 1980).

b. Nonhypoglycemic Glucoregulation

Nonhypoglycemic glucoregulation has been studied in dogs to which phlorizin has been infused. Phlorizin is the most potent non-competitive inhibitor of glucose reabsorption in the kidney tubules and its receptor has been shown to be either identical to, or part of the glucose transporter at the exterior face of the brush border membrane of the proximal tubule in dog kidney (Silverman and Black, 1965). As early as 1927, phlorizin was shown

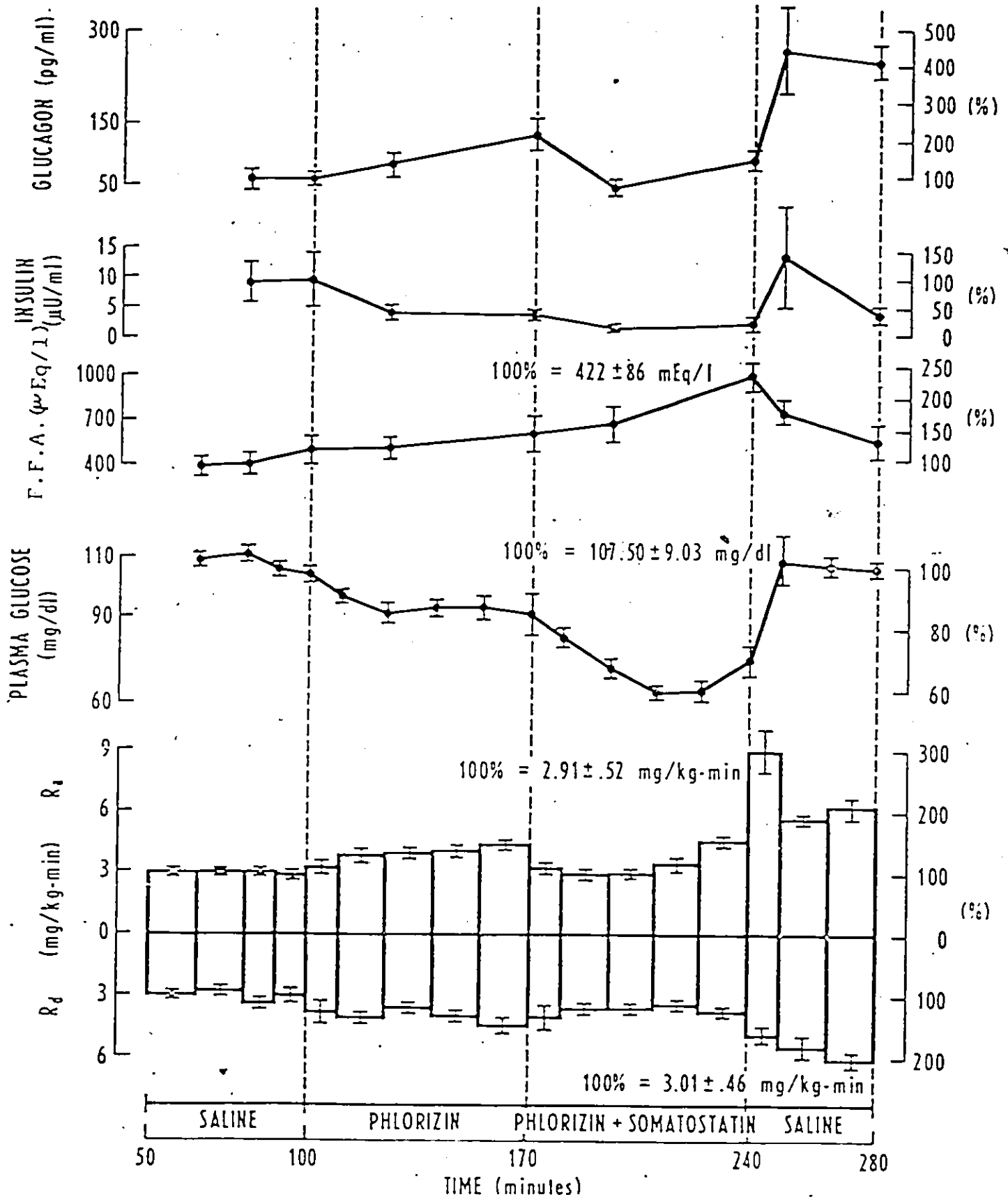
to produce prompt and persistent glucosuria without overt hypoglycemia (Nash, 1927). This glycoside appeared to be a useful tool for studying physiological processes involved in regulation of blood glucose due to the fact that the increased hepatic glucose output is due uniquely to an increased removal of glucose from blood by glycosuria (Kolodny et al., 1962) without any non-physiological increment in the level of insulin in the plasma. Phlorizin does not interfere with hepatic glucose production (Goresky et al., 1974) in doses that already produce maximal glycosuria. Fig. 1 shows the effect of an infusion of phlorizin (50 $\mu\text{g/kg.min}$) followed by that of phlorizin plus somatostatin (0.35 $\mu\text{g/kg.min}$) to 5 dogs in the postabsorptive state (Gauthier et al., 1980). Somatostatin is a tetradecapeptide that has been shown to cause acute changes in the plasma level and turnover of glucose through its effect on the endocrine system and not through any direct effect on glucose metabolism (Chideckel et al., 1975; Cherrington et al., 1977).

As it can be seen on Fig. 1, the increased loss of glucose from the plasma into the urine to a modest degree, estimated from the increment in the rate of disappearance of glucose to be approximately 1 mg/kg.min , leads to an approximately equivalent increase in the rate of hepatic glucose production: a typical example of nonhypoglycemic glucoregulation.

Figure 1. The effect of an infusion of phlorizin (50 $\mu\text{g/kg.min}$) followed by that of phlorizin plus somatostatin (0.35 $\mu\text{g/kg.min}$) on the plasma concentration of glucagon (IRG), insulin (IRI), free fatty acids (FFA) and glucose as well as on glucose appearance (Ra) and disappearance (Rd). Abscissa: time in minutes. Ordinates on the left show the variables in absolute units. On the ordinates on the right the variables are expressed as the percentage of their control values. Standard errors of the means are shown as vertical bars. For Ra, Rd and the plasma concentrations of glucose and FFA they refer to the percentage value, for IRI and IRG to absolute concentrations. Mean values of 5 experiments are shown.

Figure 1

15



As a result of this increase, the concentration of glucose in the plasma is only marginally reduced. This compensatory increase in hepatic glucose production is brought about by an increase in the concentration of glucagon in the plasma. If the rise in the concentration of glucagon is prevented by the simultaneous infusion of somatostatin, the increase in the rate of glucose release is abolished. During the simultaneous infusion of phlorizin and somatostatin the concentration of glucose in plasma drops to about 65 mg/dl, i.e. by about 40%. The infusion of 0.35 μ g/kg.min somatostatin was shown to induce a decrease of only about 15% of the basal glucose level in conscious dogs (Lickley et al. 1979).

In vivo, the α -cells seem to be very sensitive even to a marginal decrease in the level of plasma glucose. Consequently the regulation of plasma glucose concentration is very precise. This control is achieved predominantly by glucagon, and it can thus be concluded that one important physiological function of glucagon secretion is in the maintenance of plasma glucose around the set point in face of an increased rate of disappearance of glucose from the plasma.

This agrees with the concept of glucagon being an important regulator of glucose production in dog both in the postabsorptive and fasting states (Altszuler et al., 1976; Bergman, 1977; Lickley et al., 1979). A similar conclusion has also been reached in exercising dogs (Issekutz and Vranic, 1978). In their dogs, normoglycemia was maintained in face of a three-fold increase of glucose utilization when glucose rose and increments of glucose production matched the increased fuel demand of the working muscle. Plasma glucose decreased sharply however, when somatostatin was infused during exercise; plasma glucagon was suppressed and therefore glucose production did not increase adequately.

No significant increase in the plasma level of free fatty acids was observed during the infusion of phlorizin (Fig. 1). This provides indirect evidence for the absence of an increased secretion of epinephrine in nonhypoglycemic glucoregulation. This observation however needs to be supported by measurements of plasma epinephrine under identical experimental conditions. In the same way, the conclusion^s drawn about the hormonal signals of nonhypoglycemic glucoregulation in dogs in the postabsorptive state do not exclude permissive actions between these hormones as a potential contribution factor maintaining the fasting plasma glucose concentration. Indeed, although neither cortisol

(Feldman et al., 1975; Gerich et al., 1979) nor epinephrine (Walter et al., 1974) in itself play an indispensable role in the process of recovery from overt hypoglycemia, i.e. in hypoglycemic glucoregulation in man, an interesting study by Eigler et al. (1979) showed that physiologic increments in glucagon, epinephrine and cortisol interact synergistically in the normal dog so as to produce a more marked and prolonged hyperglycemia than the one expected to result from the sum of the individual effects of these hormones.

In addition to the hormonal signals which activate nonhypoglycemic glucoregulation, another question raised concerning this type of glucoregulation not yet investigated is the sources of the extra glucose released in response to the nonhypoglycemic stimulus. The mediator of nonhypoglycemic glucoregulation is glucagon, known at least in supraphysiological doses, to stimulate both glycogenolysis and gluconeogenesis (Chiasson et al., 1977). It was of interest to ascertain whether glucagon released in response to a physiological stimulus will activate both pathways of glucose production: i.e. glycogenolysis and gluconeogenesis and to assess the individual contributions of these processes in nonhypoglycemic glucoregulation.

Glycogenolysis

Liver glycogen constitutes a rapidly mobilizable source of sugar which is sufficient to cover necessary amount of glucose for 18-24 hours in man (McMurray, 1977) and dogs (Bishop et al., 1965). Fig. 2 presents schematically the regulation of glycogen metabolism. As shown on this figure, the rate-limiting enzyme for glycogen synthesis is glycogen synthetase a and the one for glycogenolysis is phosphorylase a. The activation of these two enzymes depends in turn on a further interplay of various influences such as enzymes, salts, substrate and hormones. Regulatory influences can therefore be initiated at a number of sites that directly or indirectly affect the rate-limiting enzymic reactions.

Hepatic glycogen synthesis is increased by glucose, insulin, glucocorticoids, and growth hormone. Glucose alone may increase glycogen synthesis (Bucolo et al., 1974) both in vitro and in vivo by activating synthetase. Other sugars have no such effect (Hers et al., 1970). As shown on Fig. 2, the activation of synthetase by glucose is probably brought about indirectly through its effect on phosphorylase phosphatase (Hers et al., 1970). Insulin also increases glycogen synthesis.

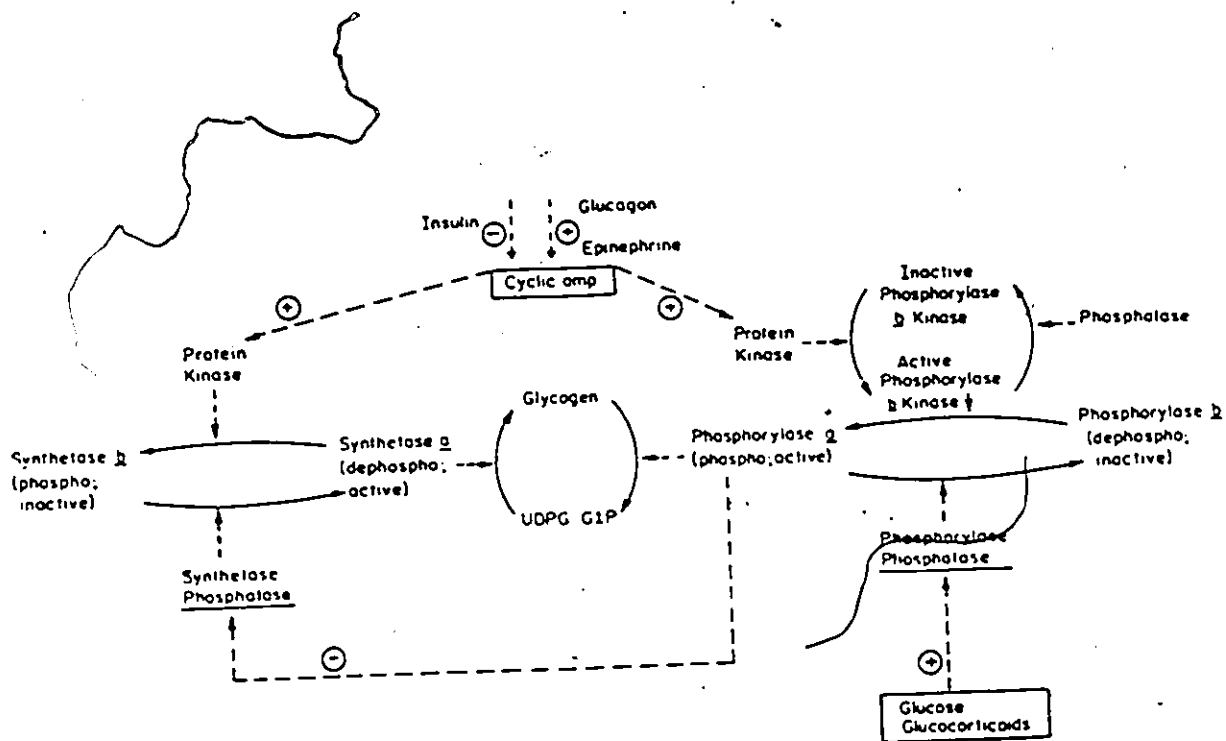


Figure 2: Regulation of glycogen metabolism in the liver (Altszuler and Finegold, 1974).

In the normal dog, the infusion of insulin, along with sufficient glucose to prevent hypoglycemia stimulates liver glycogen formation within the first hour of infusion (Bishop et al., 1965). This effect of insulin may be caused in part by a lowering of hepatic cyclic adenosine monophosphate (c-AMP) (Jefferson et al., 1968), thereby deactivating protein kinase (Fig. 2). Another possible explanation would be a decrease in the responsiveness of protein kinase to c-AMP by insulin (Shen et al., 1970). Glucocorticoids activate liver synthetase and increase the formation of glycogen indirectly by increasing synthesis of phosphorylase by glucagon, epinephrine and c-AMP (Exton et al., 1970), suggesting a "permissive" action of glucocorticoids at some site prior to c-AMP. Administration of growth hormone leads to high concentration of glycogen in the liver of rats and dogs (De Bodo and Altszuler, 1957; Bishop et al., 1967).

As shown on Fig. 2, glycogenolysis is stimulated by glucagon and epinephrine. Both agents increase the synthesis of c-AMP through their action on adenylcyclase. The intracellular c-AMP, in turn, stimulates a protein kinase, which exerts two complementary effects on glycogen: it activates phosphorylase, thereby inhibiting glycogen synthesis. Hypoglycemia stimulates glycogenolysis and increases hepatic glucose output.

It is generally assumed that glucagon and epinephrine are responsible for this effect.

Estimates of changes in the rate of glycogenolysis in the intact animal can be obtained using the protocol developed by Radziuk and Vranic (1977). By this approach, following the pre-labeling of liver glycogen with ^3H -6-glucose, changes in the concentration of this ^3H -6-glucose in the plasma with respect to time is taken as an index for a change in glycogenolysis and the concomitant release of glucose from glycogen. For this index to be valid, the specific activity (i.e. the ratio of labelled to unlabelled glycogen) of glycogen should be uniform at all parts of the molecule from which glucose is released; moreover, it should be constant during the duration of the experiment. The method does not permit the calculation of the rate of glycogenolysis in absolute terms since the specific activity of glycogen remains unknown and not necessarily uniform across the entire molecule. Nevertheless the method appears to give a useful index of glycogenolysis, indicating its suppression during hyperglycemia (Radziuk and Vranic, 1977).

As already mentioned, the second potential source of the extra glucose released in response to an infusion of phlorizin is gluconeogenesis.

Gluconeogenesis

The three main precursors for gluconeogenesis are lactate, glycerol and amino acids. Lactate is converted to pyruvate by the lactate dehydrogenase reaction and enters the gluconeogenic pathway at pyruvate carboxylase. Glycerol enters the pathway by phosphorylation to glycerol phosphate and glycerol oxidation to dihydroxyacetone phosphate, catalyzed by glycerol kinase and glycerol phosphate dehydrogenase, respectively. All of the amino acids except leucine can give rise to gluconeogenic precursors (Newsholme and Start, 1973). The points of entry into the gluconeogenic pathway of some of these amino acids as well as lactate and glycerol are shown in Fig 3 .

The estimation of the synthesis of circulating glucose from various precursors in the intact animal has been made possible through development and application of sophisticated tracer techniques over the past twenty years. For a review see Hetenyi (1981). The level of glucose in the plasma as such does not indicate the rate of glucose synthesis. For example, prolonged fasting (Cowan et al., 1969) has been shown to cause a marked decrease in the production of glucose, with a negligible

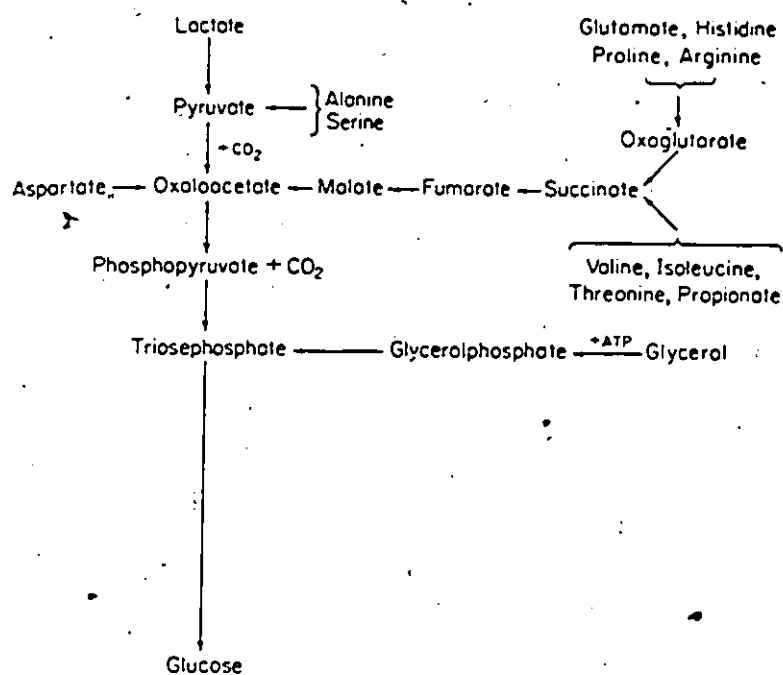


Figure 3. Entry of precursors into the pathway of gluconeogenesis: (Newsholme and Start, 1973)

if any effect on plasma glucose concentration. Generally, the protocol used for the estimation of the rate of gluconeogenesis by tracer methods is to follow the incorporation of ^{14}C -atoms into plasma glucose after the injection or during the infusion of the labeled precursor. In a dynamic steady state the ratio of the specific activities (SA) of glucose to precursor in the plasma has been used to calculate the fraction of glucose originating from the precursor. This value multiplied by the turnover rate of glucose yields the total synthesis of glucose. Dividing this by the turnover rate of the precursor, the fraction of the turnover rate of the precursor converted to glucose can be calculated. As it will be discussed later all these calculated values underestimate the true parameters.

a. The Synthesis of Glucose from Alanine

Alanine is removed from the circulation by the liver. After deamination, its carbon chain is turned into pyruvate and as such becomes a potential precursor of glucose and glycogen. Whenever alanine or lactate is used as precursor of glucose synthesis, two important facts must be taken into consideration before arriving at conclusions: (i) there is a rapid exchange of C-atoms between alanine, lactate and pyruvate (Foster et al., 1980; Hetenyi et al., 1980).

Indeed, Foster et al. (1980) calculated that in normal dogs 46% of the carbon atoms present in the alanine pool originated from lactate and that 61% of the carbon atoms in lactate have previously passed through the alanine pool, so that calculated rate of transfer to glucose from either one will also comprise C-atoms that reach glucose after passage across the pool of the other. (ii) Some ^{14}C -atoms from alanine (or lactate) are substituted by ^{12}C -atoms from Acetyl CoA in the oxaloacetic acid pool (OAA) (Krebs et al., 1966), leading consequently to an underestimation of the rate of glucose synthesis. This exchange of ^{14}C for ^{12}C -atoms can be corrected by using a factor "H" of 2.2 (Hetenyi, 1979). The Fig 4 graphically illustrates this situation.

Table 1 represents the turnover rate of alanine and the contribution of carbon atoms from plasma alanine to circulating glucose. The transfer of C-atoms from plasma alanine to glucose has been shown to be suppressed by an excess of insulin, while no effect of the splanchnic extraction of alanine was observed in dogs (Chiasson et al., 1976). Glucagon has been shown to increase the incorporation of C-atoms from plasma alanine to circulating glucose within minutes in both dogs and men (Chiasson et al., 1974; Chiasson et al., 1975). The later experiments on man (Chiasson et al., 1975) proved that glucagon in somewhat supraphysiological

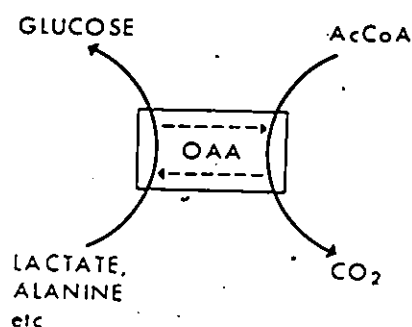


Figure 4. Schematic representation of the metabolic exchange of C atoms in the hepatic oxaloacetate (OAA) pool. The C atoms from a glucogenic substrate (e.g: lactate or alanine) on their way to glucose pass through the same pool of OAA as the C atoms from AcCoA into the Krebs cycle. The arrows represented by broken lines show the metabolic exchange of C atoms. If the OAA pool is in a steady state, the number of C atoms passing in the directions of the two arrows are taken to be equal. (From Hetenyi (1981)). Fed.Proc. in press.

Table 1. The turnover rate of alanine and the contribution of carbon atoms from plasma alanine to circulating glucose. Mean values are shown. (Modified from Hetenyi (in press))

<u>Author(s) (reference)</u>	<u>R_t alanine as mg/kg.min</u>	<u>% of glucose-carbon from alanine</u>
<u>Man</u>		
Kreisberg et al.	-	12.5
Chiasson et al.	-	1.7-6.1
Chochinov et al.	-	7.4
Bier et al.	0.35	9.0-10.9
Hall et al.	0.69	6.0
Lapidot and Nissim	0.55	-
<u>Dog</u>		
Bier et al. ¹⁴ C-tracer	1.11	-
Bier et al. ³ H-tracer	1.24	-
Chiasson et al.	-	2.2
Hall et al.	1.05	-
Balasse et al.	0.85	-
Foster et al.	1.04	8.6
Hetenyi	0.54	4.7

doses increases gluconeogenesis from alanine and that this effect is due to an increased efficiency of utilization of hepatic alanine rather than an increased extraction of alanine by the liver from the circulation. In man, fasting for 2 days caused an increased conversion of plasma alanine to glucose (Chiasson et al., 1979)

b. The Synthesis of Glucose Measured by the Recirculation of ^{14}C -atoms Into Plasma Glucose

The recirculation of ^{14}C -atoms into newly released glucose molecules will result in an underestimation of the rate of appearance of glucose. In dogs, this reincorporation of recirculation of ^{14}C -atoms infused as ^{14}C -1-glucose into the glucose molecule equals five times its reincorporation into the sixth C-atom (Forbath et al, 1969). A correction factor can thus be calculated so that: (i) the "corrected" turnover rate of glucose calculated by this approach equals that calculated with ^3H -3-glucose (Altszuler et al., 1975) and (ii) the rate of reincorporation of ^{14}C -atoms yields a rough estimate of "total" gluconeogenesis, including the Cori and alanine cycles.

c. The Synthesis of Glucose from Glycerol

Glycerol is released into the circulation by the adipocytes and is removed therefrom by the liver and to a

lesser extent by other tissues (Carmaniu and Herrera,1980). Taken up by the liver, some glycerol is converted to glucose. Table 2 shows the turnover rate of glycerol, the percent glycerol turned over transferred to glucose and the percent glucose-C from glycerol for dogs in the p.a. state as found by different investigators. In dog, fasting has been shown to increase the turnover rate of glycerol but not the rate of conversion of glycerol to glucose carbon, even though the fraction of circulating glucose originating from glycerol increased from 6.1 to 13.3% (Hall et al.,1976). Hormonal stimuli have been shown to increase the rate of turnover of glycerol in dog. Norepinephrine (Winkler,1973) and the lack of insulin in alloxan-diabetic dogs (Shaw et al.,1976) have been shown to have this effect. The rate of turnover of glycerol was found to be linearly related to its plasma level within physiological range in both man (Bortz et al.,1972) and dog (Hall,1975). The turnover rate of glycerol as a measure of lipolysis is questionable following the observation of ratio of R_t (free fatty acids) to R_t (glycerol) to be variable in humans (Björntorp et al.,1969) and dog (Shaw et al.,1975; Winkler,1969).

Table 2. The turnover rate of glycerol and the rate of carbon transfer between plasma glucose and glycerol in the postabsorptive state at rest (Modified from Hetenyi (in press)).

<u>Author(s) (reference)</u>	<u>R_t glycerol</u> <u>as mg/kg.min</u>	<u>% of glucose-carbon</u> <u>from glycerol</u>	<u>% of R_t glycerol</u> <u>transferred to glucose</u>
Steele et al.	0.49 0.05	7.0	40.0
Winkler et al.	0.49 0.04	6.3	24.0
Shaw et al.	0.25 0.02	2.5	31.0
Hall et al.	0.32 0.05	6.1	49.0

d. Gluconeogenesis in the Postabsorptive and Fasted State

The maintenance of basal level of glucose in the plasma of long fasted dogs is the evidence for an efficient long term glucoregulatory mechanism (Cowan et al., 1969). Since the fasted organism cannot rely on glycogen for more than some hours as its primary source of glucose, glucoregulation has to be achieved through an increased gluconeogenesis or/and glucose sparing.

As a matter of fact, gluconeogenesis from alanine has been shown to double in man after 2 days of fasting (Chiasson et al., 1979) and in dogs after 7 days of fasting (Hall et al., 1977). Gluconeogenesis from glycerol increased as well after 7 days of fasting in dogs (Hall et al., 1976). The rates of glucose utilization and production have been shown to be lower by about 30% after two days of fasting in cows (Kronfeld and Raggi, 1964) and 30% after 4 days of fasting in dogs (Cowan, 1969). The rapid drop in R_a and R_d of 10-20% per day comes to an apparent levelling off after 3 days of fasting. According to Cowan (1967) this represents a rapid transition from fed to fasting state, any subsequent decrease being much slower and gradual, indicating adaptation to fasting.

A decrease in the peripheral utilization of glucose mediated through a decreased insulin secretion is the most

likely mechanism mediating the regulation of glucose after 4 days of fasting. Indeed fasting is characterized by a decrease in the secretion of insulin (Cahill et al., 1966) which is significant after 3 days of fasting in dogs (DeBruijne et al., 1981). The maintenance of a basal level of glucagon also appears to be an essential factor in the regulation of blood glucose in the fasted state (Hetenyi, 1981). If any, the mediation of glucose regulation by growth hormone, cortisol and epinephrine after 4 days of fasting should be considered complementary to the insulin-glucagon ratio and in the perspective of the variations in insulin secretion.



IV - MATERIALS AND METHODS

Animals

All experiments were performed on nonanaesthetized normal healthy dogs weighing 11-16 kg, unselected as to breed or sex, and trained to stand quietly in a Pavlov stand. Animals were either in the p.a. state (16-20 h after their last meal), or in the fasted state (4 days after their last meal). The diet of the animals was a standard commercial preparation containing 26% protein, 9% fat, 61% carbohydrate and 3% fiber on a dry weight basis. Food and tap water were available ad libitum.

Procedures

Approximately 30 minutes before the experiment two plastic catheters (Clay-Adams PE-190 or 205) were inserted under local (Duracain) anaesthesia, one into the cephalic vein and the other into the inferior vena cava via a saphenous vein, so that the tip of the cannula was below the entrance of the hepatic vein. In the experiments involving prelabelling of liver glycogen, the saphenous cannula was implanted into the dog three days before the day of the experiment. The technique developed for this purpose is described in detail in Appendix I. All infusions, except ^3H -6-glucose to label liver glycogen, were given via the cephalic catheter;

blood sampling, the priming injection of tracer, as well as the infusion to label liver glycogen 3 days before the experiments, were given via the saphenous catheter.

Blood samples were transferred immediately after withdrawal into precooled plastic centrifuge tubes containing dry heparin and kept in crushed ice until centrifugation. No more than 15-20 minutes elapsed between the sampling and centrifugation which was for 10 minutes at 3,800 G.

Procedures for the isolation of the labelled metabolites, that is ^3H -3-glucose, ^{14}C -U-alanine, ^{14}C -1-glucose, ^3H -6-glucose, ^{14}C -U-glycerol and ^{14}C -glucose (New England Nuclear, Boston, M.A.) were initiated within 5 minutes after centrifugation. The concentration of plasma glucose was determined within 30 minutes after centrifugation. Plasma samples used for alanine (first series) and lactate (third series) determinations were immediately frozen at -2 to -4°C . and processed on the same day within 3 hours after centrifugation. Plasma glycerol (third series) determinations were made immediately after collection.

In all experiments, three mls of whole blood were preserved in trasylol (Sigma Chemical Co., St-Louis, U.S.A.): EDTA (Fisher Scientific, Toronto, Canada) (1:1); centrifuged at 3,800 G for 10 minutes and after the hematocrit

was read, the plasma was stored at -70°C for glucagon determination. In the third series of experiments, one ml of whole blood was preserved in glutathione (2.5 mg) (Boehringer-Mannheim, Montreal, Canada) and EGTA (1.0 mg) (Sigma Chemical Co., St-Louis, U.S.A.), mixed, centrifuged at 4800 G for 30 minutes within 30 minutes after collection, deproteinized with 2N HClO_4 (1:9), spun at 30,000 G for 30 minutes, and the plasma filtrate was stored at -70°C for epinephrine determination. An additional 1.8 ml of whole blood was poured into iced, heparinized and siliconized 2.0 ml glass tubes, centrifuged at 0°C at 2,500 r.p.m. for 10 minutes. The plasma was acidified with 1N HCl (15% of the plasma volume) and stored for ACTH determination. Finally, 3.0 ml whole blood were collected into iced and heparinized plastic tubes, centrifuged at 2,500 r.p.m. for 10 minutes and stored for cortisol determination at -70°C . Samples used for the determination of ACTH and cortisol were processed immediately after withdrawal.

Experimental Design

In all experiments a 130-minute infusion of 0.9% NaCl (phase 1) was followed by a 70 min infusion of 50 $\mu\text{g/kg.min}$ of phlorizin (phase 2). This was followed

by a final 40-min infusion of 0.9% NaCl (phase 3).

In the first series each of the five dogs used was tested in the p.a. and the fasted state. In both experiments, ^{14}C -U-alanine and ^3H -3-glucose were infused simultaneously and the turnover rates of alanine and glucose, as well as the rate of transfer of alanine to glucose (glucogenic rate), were calculated. The sequence of the two experiments on the same dog was random. At least 8 days were allowed to elapse between the two experiments. Results of experiments done on dogs in the p.a. state are reported for only 4 animals, one dog having shown excessive nervousness and atypical results over the whole experiment.

In the second series of experiments, the protocol developed by Radziuk and Vranic (1977) was followed. Briefly, in five dogs hepatic glycogen was labelled with ^3H -6-glucose three days before the day of the experiment. The experiment was carried out in the p.a. state, with ^{14}C -1-glucose infused as tracer. Two of the five dogs were also tested with an infusion of 7 mU/kg.min insulin, which replaced the phlorizin infusion. At least 8 days were allowed to elapse between the two experiments.

In the third series, two experiments were carried out on each of four dogs: one in the postabsorptive and one

in the fasted state. In both experiments, ^{14}C -U-glycerol and ^3H -3-glucose were infused simultaneously and the respective turnover rates of glycerol and glucose, as well as the rate of appearance of glucose generated from glycerol (glucogenic rate) were calculated. The sequence of the two experiments was random, and at least 8 days were allowed to elapse between them.

Blood Samples

In all experiments, 18 samples were withdrawn at 8, 15, 30, 50, 65, 80, 90, 100, 115, 130, 140, 155, 170, 185, 200, 210, 225 and 240 min after the start of tracers infusion. The concentration and specific activity of plasma alanine in the first series and glycerol and lactate in the third series, as well as those of glucose, in all three series were determined in all samples. Immunoreactive glucagon (all three series) as well as plasma ACTH and cortisol (third series) were determined at 80, 100, 130, 140, 170, 200 and 240 min after the start of tracers infusion. Plasma epinephrine in the third series was determined at $t = 100, 130, 140, 170, 200, 210$ and 324 minutes.

Chemicals

Amberlite resin IRA-400 (Cl) 100-200 mesh, Dowex



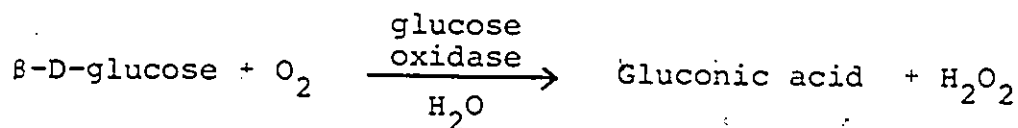
50-X8 and Amberlite resin IR-4B(OH) were obtained from BDH Laboratories, Philadelphia, U.S.A.. QAE Sephadex (stock no. Q-25-120) and L- α -glycerophosphate were obtained from Sigma Chemical Co., St-Louis, U.S.A.. All other chemicals were obtained from Fisher Scientific (Toronto, Ontario, Canada), and were of the highest grade of purity available.

Determination of the Concentration of Metabolites and Hormones in Plasma

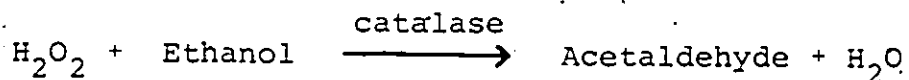
a. Assays Used in All Series of Experiments

a.1. Determination of Glucose

Glucose was determined in 10 μ l samples using a Beckman Glucose Analyzer 2 (Beckman Instruments, Inc., California, U.S.A.). The analyzer utilizes an electrode, a reagent and an electronic system that measures the rate of change in oxygen consumption when the sample is injected into the enzyme reagent solution. When the sample is injected into the enzyme reagent solution, β -D-glucose from the sample combines with dissolved oxygen from the solution, according to the reaction:



In the reaction, oxygen is consumed at the same rate as glucose reacts to form gluconic acid. The maximum observed rate has been shown to be a direct measure of the concentration of glucose originally present in the sample at the time of injection (Kadish et al., 1968). Since oxygen consumption rather than peroxide formation is measured, the resulting peroxide must be destroyed by a path not leading back to oxygen. The addition of ethanol to the reagent causes peroxide to be destroyed by catalase in the glucose oxidase, without yielding oxygen, according to the reaction



a.2. Determination of ^3H -3-glucose and ^3H -6-glucose

The concentration of tritium-labelled glucose in each sample was determined in terms of disintegrations per minute (dpm) of ionizing β radiations emitted by the isotope ^3H . 0.5 ml plasma was passed through ionic exchange resins (Reilly, 1975; Chiasson, 1974), deproteinized (Somogyi, 1945), and 1.0 ml filtrate was evaporated at 80°C in vacuo with the dry residue redissolved in one ml of distilled water. Ten ml of scintillator solution, Scintiverse (Fisher Scientific Co., Toronto, Canada), was added and, after a cooling period of 15 minutes, the mixture was counted in a Liquid Scintillation

Spectrometer Nuclear Chicago Mark-2 (Chicago, U.S.A) set on double label counting for ^3H and ^{14}C (Hetenyi and Mak, 1970). Correction for quenching and quantification of the spillover of ^{14}C into the ^3H channel, respectively were assessed by internal standardization with ^{14}C -toluene and ^3H -toluene.

a.3. Determination of Glucagon

Plasma samples were collected in a solution of trasylol (500 units/ml of blood) to protect against proteolytic loss of glucagon during collection and preparation of the plasma samples (Heding, 1969). Sodium EDTA (24 mg/ml) was used as the anticoagulant. The samples were kept in the deep freeze (-70°C) and transported in dry ice. Glucagon was determined by an immunoassay using specific antibody 30K (Faloona and Unger, 1974). Determinations of immunoreactive glucagon (IRG) were kindly done by Dr Mladen Vranic and his laboratory assistants at the University of Toronto.

b. Assays used in Individual Series of Experiments

b.1. First Series

b.1.1. Determination of Alanine in Plasma

Plasma alanine concentration was determined spectrophotometrically from the enzymic hydrogenation of NAD to NADH₂ using alanine dehydrogenase (Reilly, 1975 b). A standard curve was run for each experiment.

b.1.2. Determination of ¹⁴C-U-alanine in Plasma

¹⁴C-U-alanine was first separated from glucose and lactate by passing 1.0 ml of acidified plasma and carrier alanine (10 mg) through cationic exchange resin using the method of Reilly (1975b). The eluate was dried overnight in a Virtis Freeze-Dryer. The dried eluate was converted to pyruvate phenylhydrazone after reaction with alanine dehydrogenase to form pyruvate (Reilly, 1975b). The pyruvate phenylhydrazone crystals were then weighed, dissolved in methanol, 10 ml Scintiverse was added and the mixture was counted in a liquid scintillation counter for ¹⁴C-activity.

b.1.3. Determination of ¹⁴C-glucose in Plasma

¹⁴C-glucose contained in deproteinized filtrate was first separated from lactate using Reilly's column (Reilly, 1975a) and from alanine by shaking with Dowex 50-X8(H) and Amberlite IR-4B (OH form) resins (Chiasson, 1974).

The purified filtrate contained both ^3H and ^{14}C glucose. Less than 5% of glucose was lost during the procedure. After addition of 10 ml Scintiverse, 1.0 ml of filtrate was counted in a liquid scintillation counter set on double label counting (^3H and ^{14}C). Counting efficiency was assessed by internal standardization by ^{14}C -toluene.

b.2. Second Series

Determination of the Concentration of ^{14}C -atoms Re-incorporated into Plasma Glucose Molecule

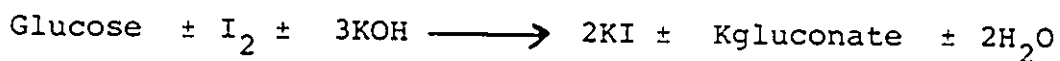
The isolation and counting of the sixth carbon atom of the glucose molecule was achieved by the method of Dunn et al. (1957). This method is based on the oxidation of the glucose molecule with periodic acid to form formaldehyde, and the subsequent reaction of the formaldehyde molecule with two molecules of methone to form methylene bis-methone precipitate. The precipitate was dried, weighed, dissolved in 10 ml Scintiverse and counted in a liquid scintillation counter for ^{14}C -activity.

b.3. Third Series

b.3.1 Determination of the Concentration of ^{14}C -glucose in Plasma

^{14}C -glucose was isolated from 0.5 ml purified

plasma filtrate (see b.1.3.) as potassium gluconate (Moore and Link, 1940) according to the following reaction:



As the reaction proceeds, the desired potassium gluconate precipitates within 50 minutes in the form of anhydrous needles, with a yield of 92% (Moore and Link, 1940). The gluconate crystals are then weighed and dissolved in 1.0 ml distilled water, 17.0 ml Scintiverse are added and the mixture is counted for ^{14}C -glucose in a liquid scintillation counter.

b.3.2. Determination of Lactate in Plasma

The enzymic determination of plasma lactate was done according to Reilly (1975a). The method is based on the reduction of NAD to NADH_2 which is coupled to the oxidation of lactate to pyruvate. Changes in optical density subsequent of the reduction of NAD were followed using a Unicam S.P. 800 Ultraviolet Spectrophotometer.

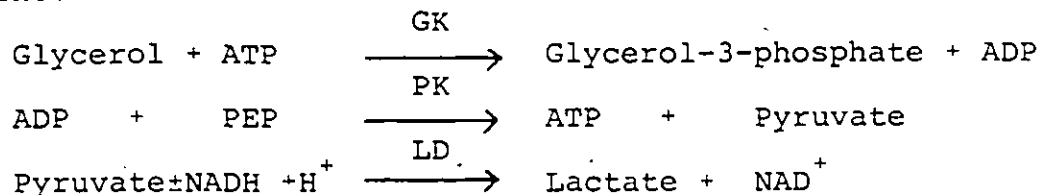
b.3.3. Determination of the Concentration of ^{14}C -lactate in Plasma

^{14}C -lactate was isolated from 0.5 ml plasma with

the help of Reilly's anionic exchange resin column (1975) with an efficiency of 97%. The sorbed ^{14}C -lactate was eluted with 16 ml 0.5M formic acid. Two ml of eluate were counted for ^{14}C -lactate activity in a liquid scintillation counter after the addition of 17 ml Scintiverse. In addition to lactate, Reilly's column has been shown to retain 97.2% of pyruvate which is not eluted with 0.5M formic acid (Reilly, 1975a) so that possible contamination of ^{14}C -lactate by ^{14}C -pyruvate is minimal.

b.3.4. Determination of Glycerol in Plasma

The concentration of plasma glycerol was determined enzymically (Garland and Randle, 1962) using a Glycerol UV Kit purchased from Boehringer Mannheim Corp. (Montreal, Canada). The assay system was modified to contain 0.35 ml buffer, 0.55 ml distilled water, 0.01 ml pyruvate kinase-lactate dehydrogenase suspension, 0.01 ml glycerokinase suspension and 0.10 ml sample. Duplicates were done for each sample. The method is based on the following reactions:



The completeness of the conversion of glycerol to glycerol-3-phosphate was assessed on a Unicam SP 800 Ultraviolet Spectrophotometer by continuous reading for 10 minutes after the addition of the enzymes. The assay was shown to be reliable within the range of 5 - 250 μ M glycerol. (see Appendix IV for details)

b.3.5. Determination of ^{14} C-U-glycerol in Plasma

^{14} C-U-glycerol was separated from ^{14} C-lactate and ^{14} C-alanine by passing 0.5 ml plasma through ionic exchange resins (Reilly, 1975; Chiasson, 1974) and subsequent deproteinization of the eluate with $\text{Ba}(\text{OH})_2$ and ZnSO_4 (Somogyi, 1945). 3.0 ml of the 1:16 filtrate were transferred to a siliconized vial and freeze-dried in a Labconco Freeze-Dry 5. The contents of vials were dissolved in 0.70 ml distilled water, of which 0.65 ml was used for the complete phosphorylation of ^{14} C-glycerol to ^{14} C-glycerol-3-phosphate according to Garland and Randle (1962).

The ^{14} C-glycerol-3-phosphate contained in the spectrophotometric cuvette was subsequently isolated by ion-exchange column chromatography according to the procedure described in Appendix IV. ^{14} C-glycerol radioactivity was determined using a liquid scintillation counter. The specific activity of glycerol was calculated according to the second

method shown in Appendix IV. This method has been shown to recover $96.6 \pm 0.2\%$ of the ^{14}C -glycerol activity.

See Appendix IV for a detailed description and the validation of the method (Gauthier and Layberry:submitted for publication).

b.3.6. Determination of Plasma Epinephrine

Plasma epinephrine was determined by the radio-enzymic assay of Sole and Hussain (1977). The assay is based on the conversion of epinephrine to its O-methylated tritiated derivative (^3H -metanephrine) by catechol-O-methyltransferase and tritiated S-adenosylmethionine. The derivative was then purified by solvent extraction and was isolated by one-dimensional, silica gel, thin layer chromatography. The spots containing the O-methylated compound were scraped directly into vials and were counted in a liquid scintillation counter. Determinations of epinephrine were kindly done by Dr Mladen Vranic and his laboratory assistants at the University of Toronto. Samples were transported in dry ice to Toronto.

b.3.7 Determination of Cortisol in Plasma

Plasma cortisol determination was carried out using the method of Silber et al. (1958). After extraction in

methylene chloride, a mixture of 65% conc. H_2SO_4 and 35% absolute ethanol was added to the extract and the resulting fluorescence measured against standards on an Aminco-Bowman Spectrophotofluorometer using an excitation wavelength of 565 nm and an emission wavelength of 525 nm. Determinations of cortisol were kindly done by Dr John S. Cowan and his laboratory assistants at the University of Ottawa.

• b.3.8. Determination of ACTH in Plasma

Plasma ACTH was determined by a bioassay originally described by Sayers et al. (1971) as modified by Cowan et al. (1974). The assay is based on the increased production of corticosterone by suspensions of rat adrenal cells in response to the addition of ACTH. Plasma samples were compared to standards prepared in trypsin deactivated dog plasma. The determination of corticosterone was achieved by the method described in b.3.7. for cortisol, and correction was applied for the presence of endogenous cortisol. Determinations of ACTH were kindly done by Dr John S. Cowan and his laboratory assistants at the University of Ottawa.

Calculations

a. General

a.1. Rates of Appearance and Disappearance of Glucose

The rate of appearance (Ra) was taken to be identical with the hepatic glucose production, and the rate of disappearance (Rd), with the overall glucose utilization plus glucosuria, if any. The primed tracer infusion method originally described by DeBodo et al. (1963) and modified by Cowan and Hetenyi (1971) was used to calculate Ra and Rd. This method assumes a one compartment glucose system having different mechanisms controlling the production rate (input) by the liver and its disappearance (output) in the various tissues of the body. The rate of appearance of glucose is calculated as

$$Ra = (F - (p \times C_t \times V \times dSA/dt)) \times 1/SA_t$$

where Ra is the rate of appearance of glucose (mg/kg.min), F is the infusion rate of labeled glucose (5.55×10^5 dpm/min), p is the "pool fraction" taken to be equal to 0.65 (Cowan and Hetenyi, 1971; Radziuk et al., 1978), V is the glucose distribution volume (ml) and was taken to be equal to 24% of the body weight of the animal expressed in milliliters (DeBodo et al., 1963), C_t is the plasma glucose concentration

(mg/ml) at time t and SA_t is the specific activity (dpm/mg) at time t . The rate of disappearance of glucose was calculated as

$$R_d = R_a - (p \times V \times dC_t/dt)$$

where R_d is the rate of disappearance of glucose (mg/kg.min) and the other symbols are as above. The metabolic clearance rate of glucose (MCR) was calculated as the ratio of the rate of disappearance of glucose (R_d) to the plasma glucose concentration (C).

Polynomials were fitted to the SA and plasma concentration vs time curves of plasma glucose. A tricubic spline algorithm with smoothing (Späth, 1974) was used for this purpose. All calculations were carried out on an IBM 375/65 Computer by a single program.

a.2. Rates of Appearance and Disappearance of Alanine

The methods used to calculate R_a and R_d were the same as used for glucose, except for F being equal to 8.88×10^5 dpm/min and the pool fraction and distribution volume were taken to be 1.0 and 65% of the body weight (Hall et al., 1977) respectively.

a.3 Rates of Appearance and Disappearance of Glycerol

The same calculations were carried out as above (see section a.1. and a.2.) but F for ^{14}C -U-glycerol equalled 8.88×10^5 dpm/min and the pool fraction and distribution volume were taken to be 1.0 and 66.8% of body weight (Hall et al., 1976) respectively.

a.4 Statistical Analysis

The changes between two phases within an experiment, the changes between the two nutritional states within the same phase and the increments between the first and second phase in the two nutritional states were compared. A complete analysis of variance (Snedecor, 1956) was carried out with the original data on plasma levels of the measured hormones and with areas under the curves as measured by planimetry for all other (time dependent) parameters. The F and P values calculated for the differences were taken as measures of significance.

b. Calculations Used in Individual Series of Experiments

b.1. First Series

Glucogenic Rate, Fraction of Circulating Glucose Coming from Gluconeogenesis and Fraction of the Utilized Precursor Going into Glucose

In the first series of experiments, the calculation of the rate of transfer of carbon atoms from plasma alanine to glucose is schematically represented on Fig 5. R^*_1 and R^*_2 represent the rate of infusion of ^{14}C -U-alanine and ^3H -3-glucose as dpm per minute; $\text{SA}(^{14}\text{C}\text{-alanine})$ refers to the specific activity of alanine in plasma and $\text{SA}(^{14}\text{C}\text{-glucose})$ and $\text{SA}(^3\text{H}\text{-glucose})$ refer to the specific activities of glucose in plasma, with respect to ^{14}C coming from the infused ^{14}C -U-alanine and ^3H coming from the infused ^3H -3-glucose. Since both ^{14}C -U-alanine and ^3H -3-glucose are infused simultaneously into the same dog, the fraction (F) of R^*_1 getting into glucose from alanine can be calculated from the following relationship:

$$\text{Turnover rate of glucose} = \frac{R^*_2}{\text{SA}(^3\text{H}\text{-glucose})} = \frac{F \times R^*_1}{\text{SA}(^{14}\text{C}\text{-glucose})}$$

$$\text{That is} \quad F = \frac{R^*_2 / \text{SA}(^3\text{H}\text{-glucose})}{R^*_1 / \text{SA}(^{14}\text{C}\text{-glucose})}$$

The loss of ^{14}C -atoms on route from alanine to glucose by "metabolic exchange" between ^{12}C and ^{14}C atoms in the hepatic oxaloacetate pool (Krebs et al., 1966) was compensated for by multiplying the product of $F \times R^*_1$ by a factor equal to 2.2 (Hetenyi, 1979). Therefore the rate of gluconeogenesis

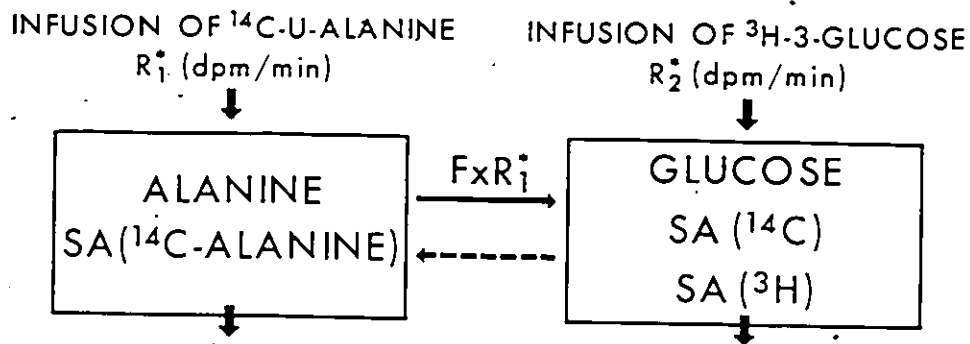


Figure 5. Model used in the calculation of the fluxes of carbon-atoms across and between the plasma alanine and glucose pools.

(R), i.e. the rate of transfer of C-atoms (usually expressed as mg Carbon/min) from alanine present in the plasma or in equilibrium with plasma alanine is

$$R = \frac{F \times R^*_1 \times H}{SA(^{14}\text{C-alanine})}$$

The fraction of C-atoms in circulating glucose arising from gluconeogenesis was calculated as the ratio of the gluconeogenic rate over the rate of appearance of glucose in commensurable units. Finally, the fraction of mobilized alanine converted to glucose was calculated as the ratio of the gluconeogenic rate to the rate of disappearance of alanine.

b.2. Second Series

b.2.1. Gluconeogenic Rate (recirculation of ^{14}C -atom into glucose)

Fig 6 shows the model used in the calculation of the glucogenic rates in the second series of experiments. R^*_1 and R^*_2 represent the rate of infusion of ^{14}C -1-glucose and the endogenous infusion of ^3H -6-glucose as dpm per minute response; $SA(^3\text{H-6-glucose})$ and $SA(^{14}\text{C-6-glucose})$ refer to the specific activities of glucose obtained from the isolation of the 6th carbon atom from plasma glucose molecule with its

INFUSION OF ^{14}C -1-GLUCOSE

ENDOGENOUS (HEPATIC) INFUSION
OF ^3H -6-GLUCOSE

R^*_1 (dpm/min)

R^*_2 (dpm/min)

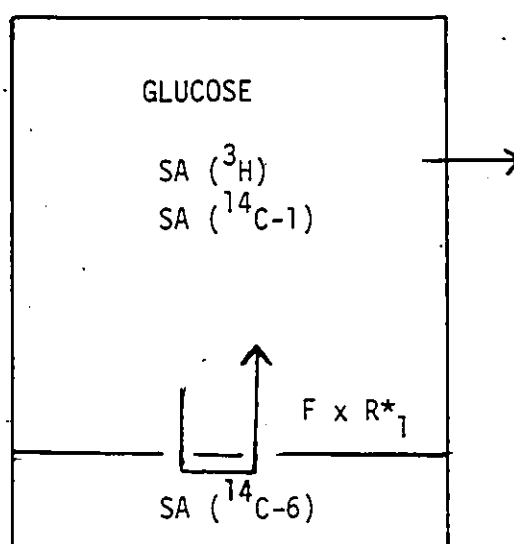


Figure 6. Model used in the calculation of the recirculation of carbon-atoms within the glucose molecule and the flux of glucose from the hepatic glycogen pool to plasma.

attached H-atom as HCHO (Reichard et al., 1963). The ^3H -activity originated from hepatic glycogen previously labelled with ^3H -6-glucose, the ^{14}C -activity arose from recirculation of ^{14}C -atoms originally on the first carbon position into glucose in plasma after passage through the Cori and/or the alanine-glucose cycles (Lickley et al., 1979). The $\text{SA}(^{14}\text{C}-1)$ is the specific activity of the first C-atom of glucose and was calculated as the difference between total ^{14}C -radioactivity in plasma glucose minus five times the ^{14}C -activity on the 6th C-atom (Forbath et al., 1969). Since ^{14}C -1-glucose is infused continuously, the fraction (F) of R^*_1 recirculating into glucose from ^{14}C -1-glucose can be calculated from the following relationship:

$$\text{Turnover rate of glucose} = \frac{\text{R}^*_1}{\text{SA}(^{14}\text{C}-1)} = \frac{\text{F} \times \text{R}^*_1}{\text{SA}(^{14}\text{C}-6) \times 5}$$

$$\text{and } \text{F} = \frac{\text{SA}(^{14}\text{C}-6) \times 5}{\text{SA}(^{14}\text{C}-1)}$$

The results were expressed as the normalized values for basal $\text{F} \times \text{R}^*_1$.

b.2.2 Glycogenolytic Rate

This has been calculated in two different ways, depending whether dC/dt was large or not.

b.2.2.1. One-Compartment Model

In the experiments where phlorizin was infused and the concentration of glucose in the plasma dropped only slightly, the glycogenolytic rate (ggl) was calculated from the Ra of ^{14}C -l-glucose and the specific activity (SA) of ^3H -6-glucose according to the following formula, which is basically the one-compartmental model of Steele et al. (1956):

$$\text{ggl}_t \equiv (\text{Ra}_t \times \text{SA}_t) - (p \times V \times C_t \times d\text{SA}/dt)$$

p , V and C have already been defined earlier.

b.2.2.2. Two-Compartment Model

In experiments where insulin was infused and the rate of fall in plasma glucose was rapid, the use of one-compartmental model led to contradictory results for ggl during the first 30 minutes after the start of the infusion of insulin. A double compartmented approximation of the system as described by Radziuk et al. (1978) was thus adopted to gain greater precision in spite of these sudden and large deviations from steady state in glucose concentration. The two-compartmental

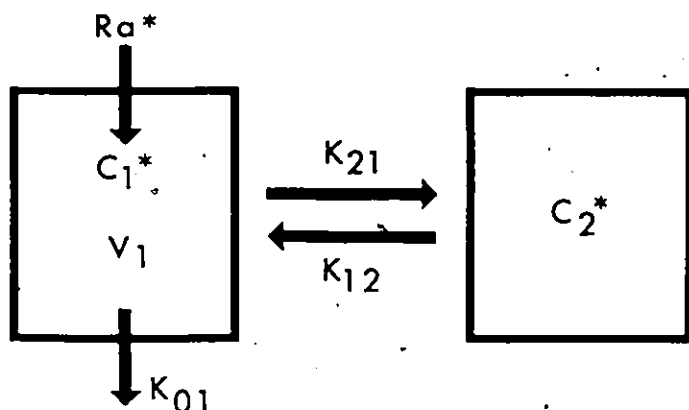
model is shown on Fig 7 . Ra^* is the rate of appearance of ^3H -6-glucose expressed as dpm per minute which is endogenously infused by the liver. C^*_1 is the concentration of ^3H -6-glucose in compartment 1 in dpm per ml; V_1 is the distribution volume of compartment 1. C^*_2 is the concentration of ^3H -6-glucose in compartment 2 in dpm per ml. K_{01} is the rate of tracer going out of compartment 1. K_{12} and K_{21} are the rates of exchange of tracer between the two compartments (min^{-1}) and have been calculated from a double exponential curve fit of data obtained following a bolus injection of labelled glucose to a dog in the p.a. state. Detail of the calculations for V_1 , K_{12} and K_{21} are shown in Appendix III.

b.3. Third Series

b.3.1. Glucogenic Rate, Fraction of Circulating Glucose Coming from Gluconeogenesis and Fraction of the Utilized Precursor going into Glucose

The calculation of the rate of transfer of carbon atoms from plasma glycerol to glucose in the third series of experiments is schematically represented on Fig 8 .

R^*_1 and R^*_2 represent the rate of infusion of ^{14}C -U-glycerol and ^3H -3-glucose as dpm per minute; $SA(^{14}C\text{-glycerol})$ refers



$$Ra^*(t) = V_1 \cdot \left[\frac{dC_1^*}{dt} + (K_{21} + K_{01}(t))C_1^* - K_{12}C_2^* \right]$$

Ra^* = GLYCOGENOLYSIS (dpm.min⁻¹)

C_1^* = CONCENTRATION OF ³H-6-GLUCOSE IN COMPARTMENT 1.
(dpm.ml⁻¹)

V_1 = DISTRIBUTION VOLUME OF COMPARTMENT 1. (ml)

C_2^* = CONCENTRATION OF ³H-6-GLUCOSE IN COMPARTMENT 2.
(dpm.ml⁻¹)

K_{12}, K_{21} = RATES OF EXCHANGE OF TRACER BETWEEN THE TWO
COMPARTMENTS (min⁻¹)

K_{01} = RATE OF TRACER GOING OUT OF COMPARTMENT 1.

Figure 7. Schematic representation of the double-compartmented model of the glucose system.

INFUSION OF ^{14}C -U-GLYCEROL INFUSION OF ^3H -3-GLUCOSE

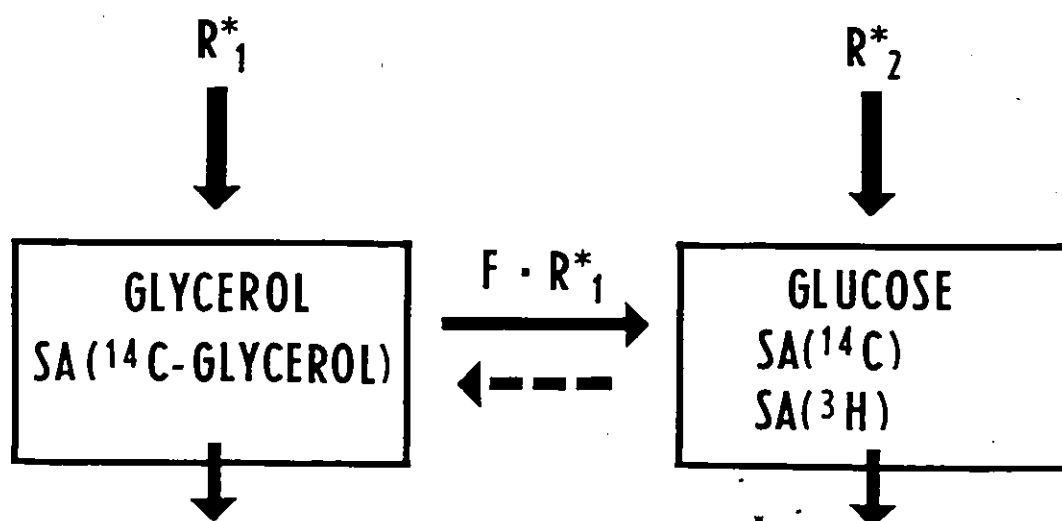


Figure 8. Model used in the calculation of the fluxes of carbon atoms across and between the plasma glycerol and glucose pools.

to the specific activity of glycerol in plasma and SA (^{14}C -glucose) and SA(^3H -glucose) refer to the specific activities of glucose in plasma, with respect to the ^{14}C coming from the infused ^{14}C -U-glycerol and ^3H as a result of the infusion of ^3H -3-glucose. Since both ^{14}C -U-glycerol and ^3H -3-glucose are infused simultaneously into the same dog, the fraction (F) of R^*_1 getting into glucose from glycerol can be calculated from the following relationship:

$$\text{Turnover rate of glucose} \equiv \frac{R^*_2}{\text{SA}(^3\text{H-glucose})} \equiv \frac{F \times R^*_1}{\text{SA}(^{14}\text{C-glucose})}$$

That is

$$F \equiv \frac{R^*_2 / \text{SA}(^3\text{H-glucose})}{R^*_1 / \text{SA}(^{14}\text{C-glucose})}$$

Therefore the rate of gluconeogenesis (R), i.e. the rate of transfer of carbon atoms to glucose from glycerol present in the plasma or in equilibrium with plasma glycerol is

$$R \equiv \frac{F \times R^*_1}{\text{SA}(^{14}\text{C-glycerol})}$$

The fraction of C-atoms in the circulating glucose arising from gluconeogenesis and the fraction of mobilized glycerol

converted to glucose were calculated in the same way as for the first series of experiments.

b.3.2. The Fraction of Circulating Glucose Coming from Lactate

The Fraction of Circulating Glucose coming from lactate has been calculated only under dynamic steady state conditions (i.e. between 100 and 130 minutes) for each dog in the postabsorptive state. Results are reported in Table 4 .

b.3.3. Statistical Analysis

The changes between two phases within an experiment were compared. A complete analysis of variance (Snedecor, 1956) was carried out with original data on plasma levels of the measured hormones and with areas under the curves as measured by planimetry for all other (time dependent) parameters. The F and P value calculated for the difference were taken as measures of significance. The paired Student's t test was used for the statistical assessment of the differences in basal values as well as the differences in increments between the first and second phase between the two nutritional states in the same dogs. The level of significance (p) was assessed by the "one-tail" criterion (Colton,1974) for the turnover rates of glucose and glycerol since the respective hypotheses to be tested were whether the turnover rate of glucose is less and whether the turnover rate of glycerol is more in the fasted state.

V - RESULTSFirst Series: Gluconeogenesis from Alanine in Nonhypoglycemic Glucoregulation.

The results of the first series of experiments are shown in Fig. 9 and 10. Original values for plasma alanine concentration are reported in Table 5. In the p.a. state the average R_a (glucose) was 3.23 ± 0.16 mg/kg. min., 7.2% of which arose by gluconeogenesis from circulating alanine yielding a rate of synthesis of glucose from alanine of 0.238 ± 0.024 mg/kg.min. During the infusion of phlorizin the concentration of glucose in plasma was decreased by 15% ($F = 128.5$, $P < 0.005$). The clearance of glucose from the plasma was also increased ($F = 170.6$, $P < 0.005$) reflecting glycosuria. In an apparent response to this the concentration of glucagon (IRG) in the plasma rose ($F = 5.16$, $P < 0.05$) as did the rate of glucose production ($F = 38.04$, $P < 0.005$) together with the fraction of glucose arising from alanine. However, the increase became significant only after 30 minutes of infusion of phlorizin ($F = 12.36$, $P < 0.01$). Finally, the fraction of the utilized alanine converted to glucose was elevated significantly from 26 to 58% during the infusion ($F = 78.84$, $P < 0.005$).

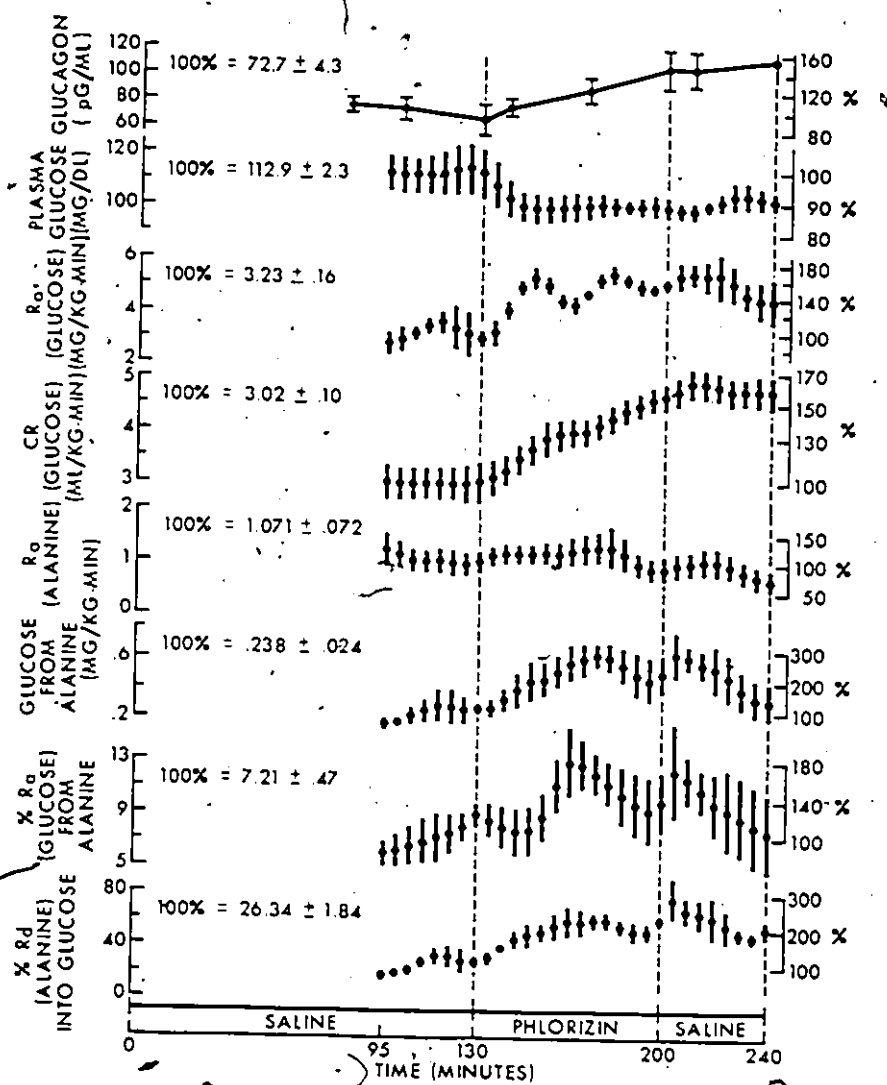
Figure 9: Effect of infusing phlorizin at $50 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$

in dogs in the p.a. state, on plasma concentration of immunoreactive glucagon (IRG), plasma glucose concentration, rate of appearance (Ra) and clearance rate (CR) of glucose, rate of appearance (Ra) of alanine, rate of formation of glucose from alanine, fraction of circulating glucose arising from alanine and the fraction of utilized alanine-C transferred to glucose. Abscissa: time in minutes.

Ordinates on the left: variables in absolute units.

Ordinates on the right: variables expressed as percentage of their control values. Standard errors of the means are shown as vertical bars. (n=4)

Figure 9



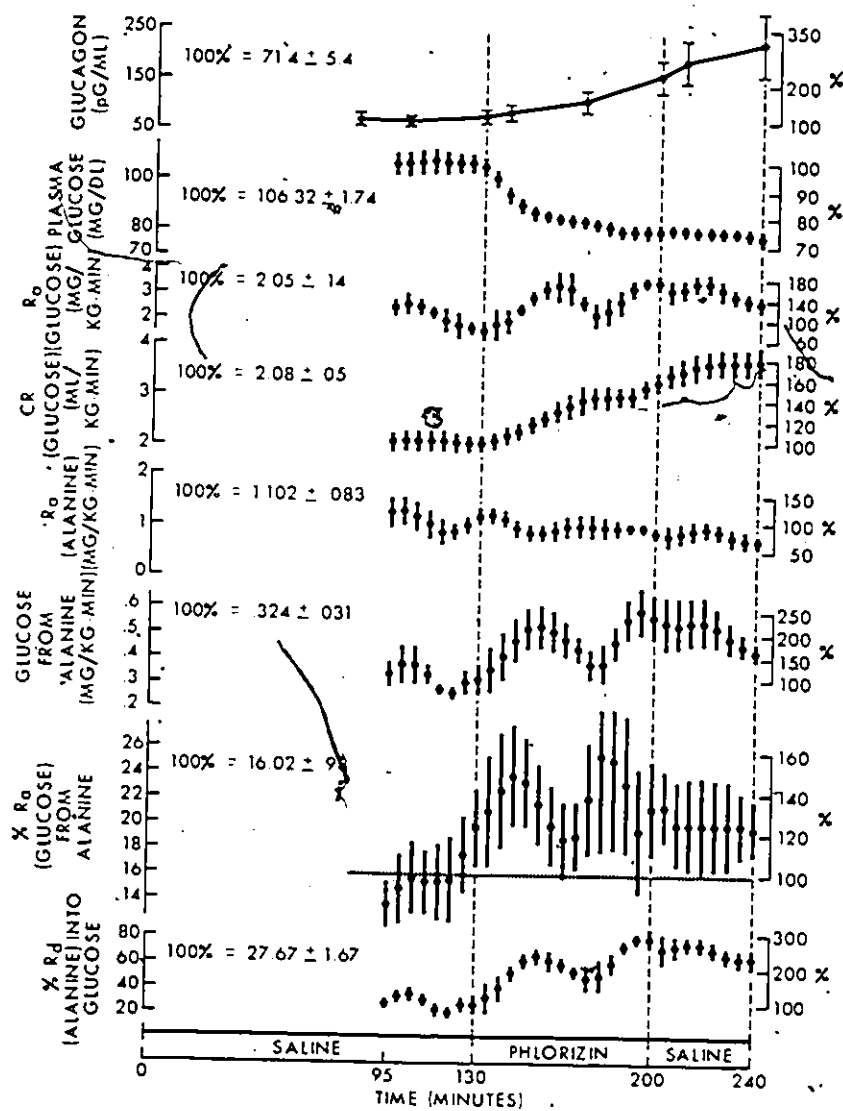
As shown in Fig. 10 in fasted dogs the mean rate of glucose production was 2.05 ± 0.14 mg/kg. min., significantly lower than in the p.a. state ($F = 16.08$, $P < 0.01$). The clearance of glucose from plasma was also lower ($F = 13.94$, $P < 0.01$). 0.324 ± 0.031 mg/kg.min of glucose was synthesized from alanine accounting for $16.02 \pm 0.98\%$ of plasma glucose. The difference in basal fraction of circulating glucose arising from alanine between p.a. and fasted dogs is significant ($F = 17.53$, $P < 0.01$). During the infusion of phlorizin, the concentration of glucose in plasma fell by 25%, the decrease was significant ($F = 49.0$, $P < 0.005$) and also significantly larger than observed in the p.a. state ($F = 11.46$, $P < 0.05$). Both the rate of appearance of glucose and the clearance of glucose from plasma increased significantly during the infusion of phlorizin ($F = 5.58$, $P < 0.05$ and $F = 299.1$, $P < 0.005$ resp.). The increase in the rate of appearance of glucose was the same as observed in the p.a. state, the increase in the clearance was less ($F = 9.90$, $P < .05$). The concentration of IRG rose ($F = 13.37$, $P < 0.01$) and the rise was somewhat larger than in the p.a. state ($F = 4.53$, $P < 0.05$). Original values for plasma alanine concentration are reported in Table 5. There was no change in the turnover rate of alanine. The rate of glucose synthesis from plasma alanine was increased significantly ($F = 20.66$, $P < 0.05$), together with the fraction of plasma glucose arising from alanine ($F = 10.05$, $P < 0.05$).

Figure 10. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the fasted state on plasma concentration of immunoreactive glucagon (IRG), plasma glucose concentration, rate of appearance (Ra) and clearance rate (CR) of glucose, rate of appearance (Ra) of alanine, rate of formation of glucose from alanine, fraction of circulating glucose arising from alanine and the fraction of utilized alanine-C going into glucose. Abscissa: time in minutes.

Ordinates on the left: variables in absolute units.

Ordinates on the right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. (n=5)

Figure 10



Under basal conditions the fraction of utilized alanine converted to glucose was about the same as in the p.a. state and increased significantly during the infusion of phlorizin ($F = 53.50$, $P < 0.005$) to the same extent as in the p.a. state. The statistical analysis of the results of these experiments is given in Appendix IV and in Tables 6 to 35.

Second Series: Glycogenolysis in Nonhypoglycemic and Hypoglycemic Glucoregulation.

The results of the second series of experiments are shown in Fig. 11 and 12. As shown in Fig. 11, plasma glucagon and glucose concentrations, as well as the rate of appearance (R_a) and clearance rate (CR) of glucose, responded to phlorizin in the same way as the first series (compare with Fig. 9). Calculated from the specific activity of ^3H -6-glucose and the rate of appearance of ^{14}C -1-glucose, the rate of production of glucose from hepatic glycogen increased significantly ($F = 16.87$, $P < .01$) within 10 minutes and stayed at a level twice the basal. As measured from the recirculation of ^{14}C -atoms from the first into the 6th carbon atom of glucose, gluconeogenesis was found to have increased significantly during the infusion of phlorizin ($F = 26.32$, $P < .01$). As shown in Table 3, the calculated rates of glucose appearance did not differ significantly when computed on the basis of a single compartmental (Steele et al., 1956) or a two-compartmental (Radziuk et al., 1978) model.

Figure 11. Effect of infusing phlorizin at $50 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in dogs in the p.a. state on plasma concentration of immunoreactive glucagon (IRG), plasma glucose concentration, rate of appearance (Ra) and clearance rate (CR) of glucose, rate of glycogenolysis and rate of recirculation of ^{14}C -atom into glucose. Abscissa: time in minutes. Ordinates on left: variables in absolute units. Ordinates on right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. The rate of glycogenolysis and the rate of recirculation of ^{14}C are expressed as normalized values for their respective basal value only. (n=5)

Figure 11

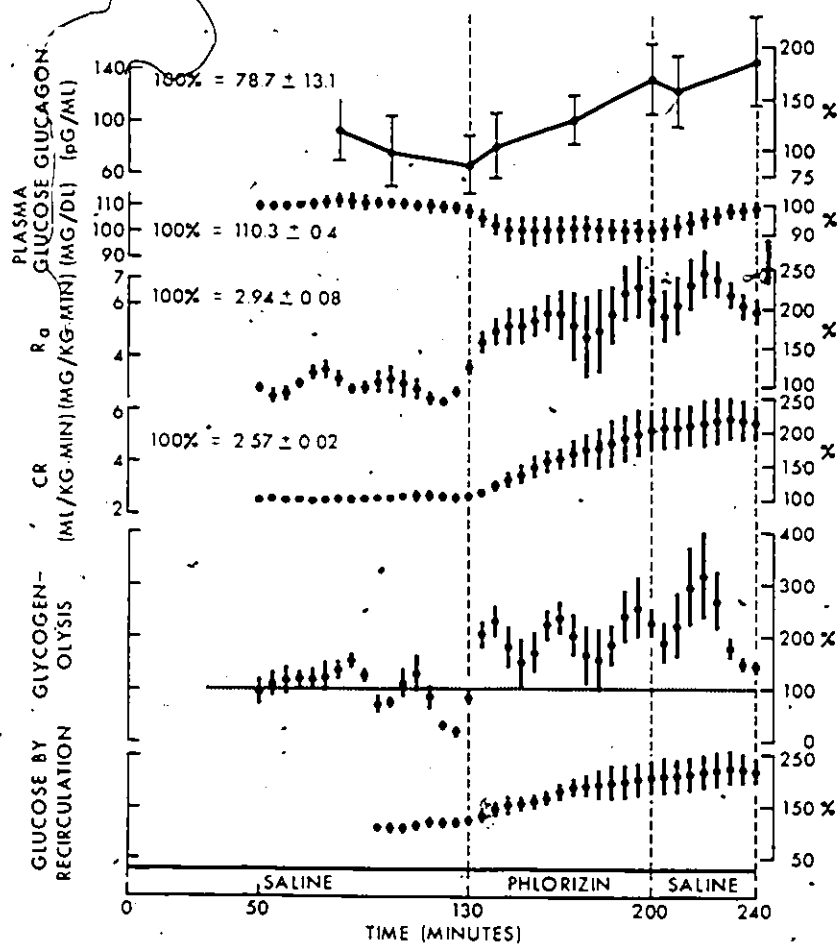


TABLE 3

The rate of appearance of glucose in dogs calculated before, during and after the infusion of phlorizin between the one and two-compartmental model.

ANALYSIS OF VARIANCE

Source of variation	df	SS	MS	F	P
<u>Before infusion</u>					
Between models	1	0.008	0.008	0.032	n.s.
Between dogs	4	25.56	6.392	25.28	0.01
Interaction	4	0.204	0.051	0.202	
Error	150	37.93	0.252		
<u>During infusion</u>					
Between models	1	7.056	7.056	3.178	n.s.
Between dogs	4	294.36	73.59	33.147	0.01
Interaction	4	1.098	0.274	0.123	
Error	130	288.61	2.22		
<u>After infusion</u>					
Between models	1	2.903	2.903	3.752	n.s.
Between dogs	4	175.05	43.761	56.564	0.01
Interaction	4	0.845	0.211	0.273	
Error	70	54.16	0.773		

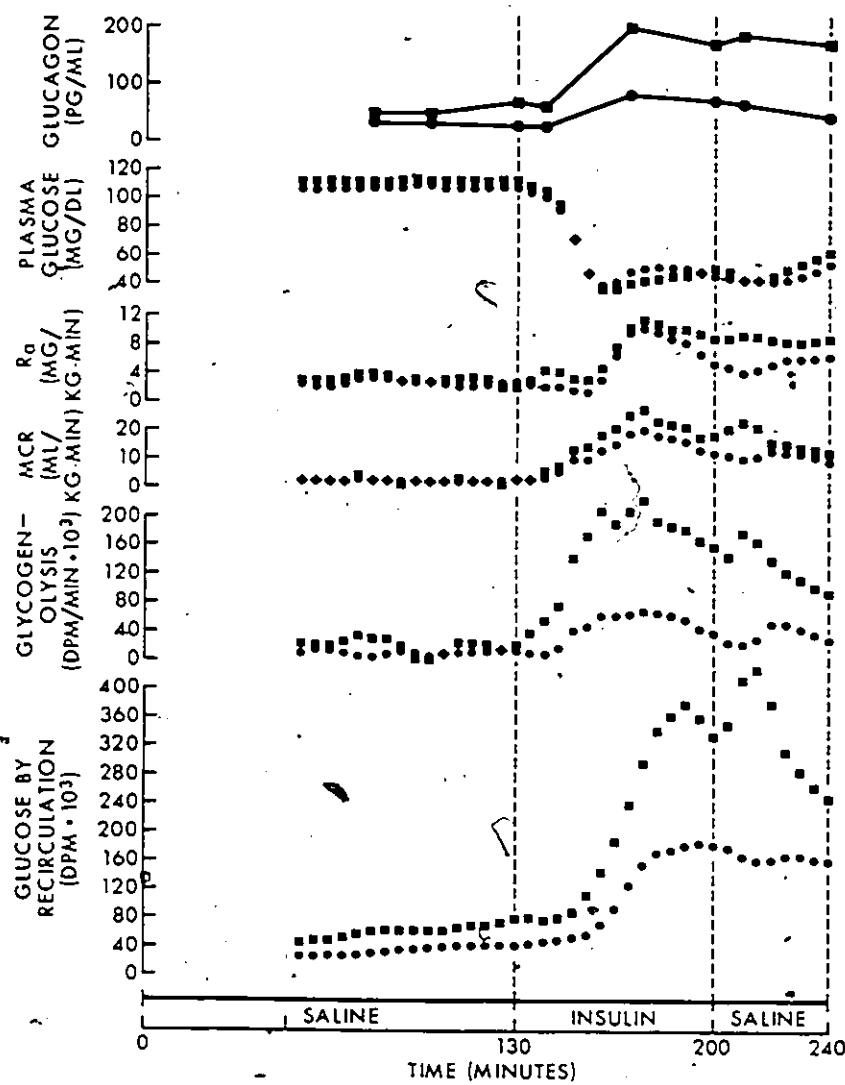
The effects of insulin-induced hypoglycaemia on 2 dogs in the postabsorptive state are shown in Fig. 12. Insulin reduced the level of plasma glucose to about 45 mg/dl. This hypoglycaemia, which was due to an 8-fold increase in the metabolic clearance rate of glucose (MRC), elicited a 4-fold increase in the rate of appearance of glucose (Ra). An 3- to 4-fold increase in the concentration of plasma immunoreactive glucagon (IRG) was also observed. Expressed as dpm of ^3H -6-glucose appearing in the plasma per minute, the rate of glycogenolysis was increased by 6- to 10-fold in 2 dogs, infused with insulin, while the rate of gluconeogenesis, expressed as dpm ^{14}C recirculating from the first to the sixth C-atom in the glucose molecule per minute, increased by a factor of 4.5. The statistical analysis of the results of these experiments is given in Appendix IV and on Tables 36 to 41.

Third Series: The Role of Glycerol and Epinephrine, ACTH and Cortisol in Nonhypoglycemic Glucoregulation.

The results of the third series of experiments are shown in Fig. 13 to 18. The results of the experiments carried out on dogs in the p.a. state are summarized in Fig. 13 to 15. Under the near-steady-state conditions preceding the infusion of phlorizin, the rate of turnover

Figure 12. Effect of infusing insulin at $7 \text{ mU.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state on plasma concentration of immuno-reactive glucagon (IRG), plasma glucose concentration, rate of appearance (Ra) and metabolic clearance rate (MCR) of glucose, rate of glycogenolysis and rate of recirculation of ^{14}C -atom into glucose. Abscissa: time in minutes. Ordinates: variables in absolute units. Ra and the rate of glycogenolysis were analyzed on the basis of a 2-compartmented model of the glucose system.

Figure 12



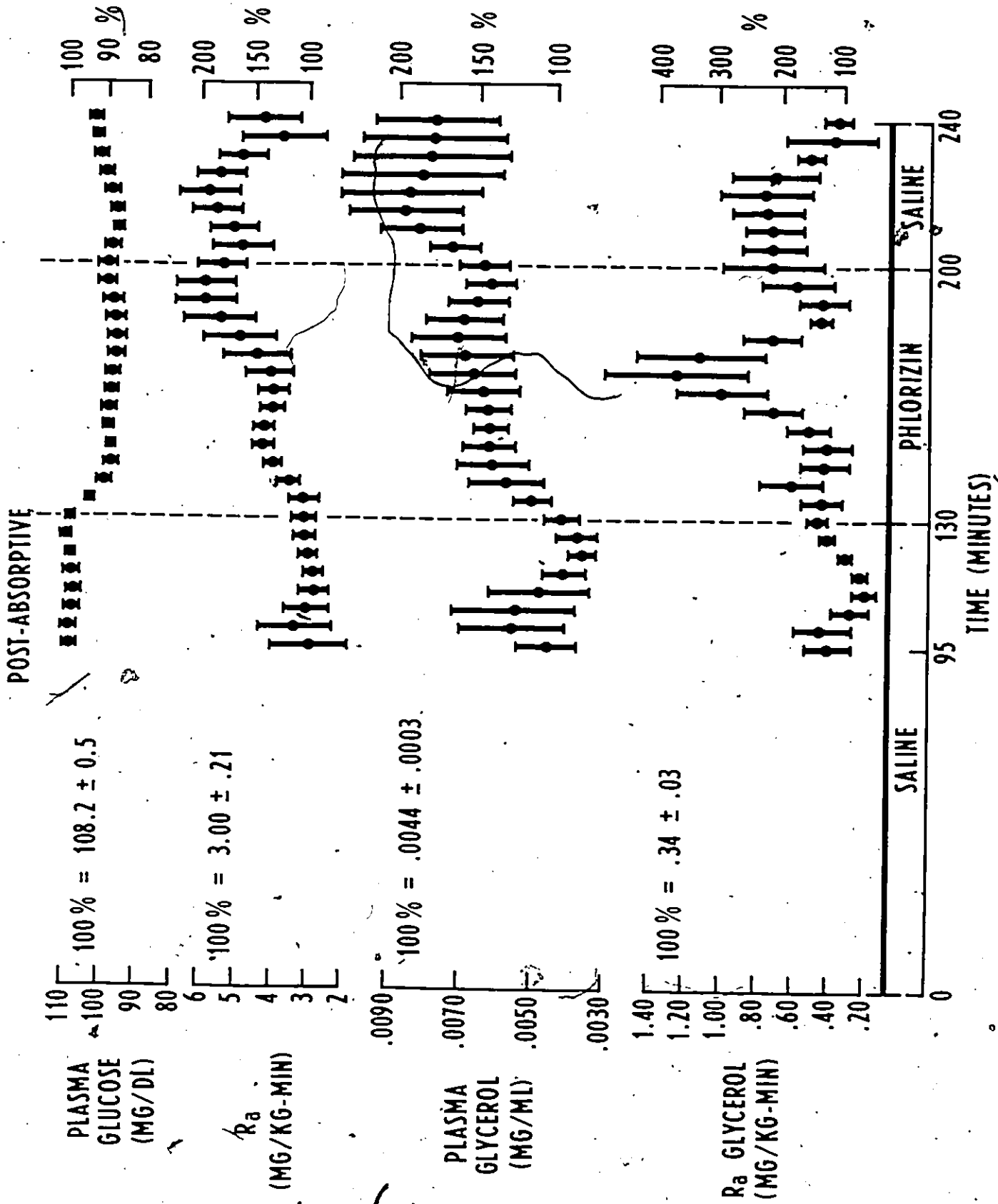
of glycerol was 0.34 ± 0.03 mg/kg.min and 0.108 ± 0.009 mg/kg.min. of glucose was formed from glycerol. This amount corresponds to $34.07 \pm 2.55\%$ of the turnover rate of glycerol and contributes $3.58 \pm 0.31\%$ of the glucose released into the plasma. Plasma lactate also became labelled to a significant extent (fig. 14).

During the infusion of phlorizin the concentration of IRG in the plasma, as well as the rate of glucose production increased, and the concentration of glucose in plasma decreased to a small extent. These changes agree very closely with our earlier findings (Gauthier et al. 1980; Gauthier et al., in press). The rate of glycerol release into the plasma increased, but this increase was transient and there was large variability between results in individuals. Nevertheless an analysis of variance revealed the increment to be significant ($F = 11.2$, $P < .05$). The concentration of glycerol in plasma also tended to increase during the infusion of phlorizin, but this change was not statistically significant ($F = 2.784$, $P > 0.10$). Following a somewhat similar time course, the rate of gluconeogenesis from glycerol also significantly increased, reaching a peak 440% its basal level ($F = 35.92$, $P < .005$). Concurrently, the fraction of plasma glucose arising from glycerol also rose, reaching a peak almost three times the basal level ($F = 24.40$, $P < .01$).

Figure 13. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state, on plasma glucose concentration, rate of appearance (Ra) of glucose, plasma glycerol concentration and rate of appearance (Ra) of glycerol.

Abscissa: time in minutes. Ordinates on the left: variables in absolute units. Ordinates on the right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. (n=4)

Figure 13



79

Figure 14. Effect of infusing phlorizin at $50 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in dogs in the p.a. state, on the rate of formation of glucose from glycerol, the fraction of circulating glucose arising from glycerol, the fraction of utilized glycerol-C transferred to glucose, the specific activity of lactate and the plasma lactate concentration. Abscissa: time in minutes. Ordinates on the left: variables in absolute units. Ordinates on the right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. (n=4)

Figure 14

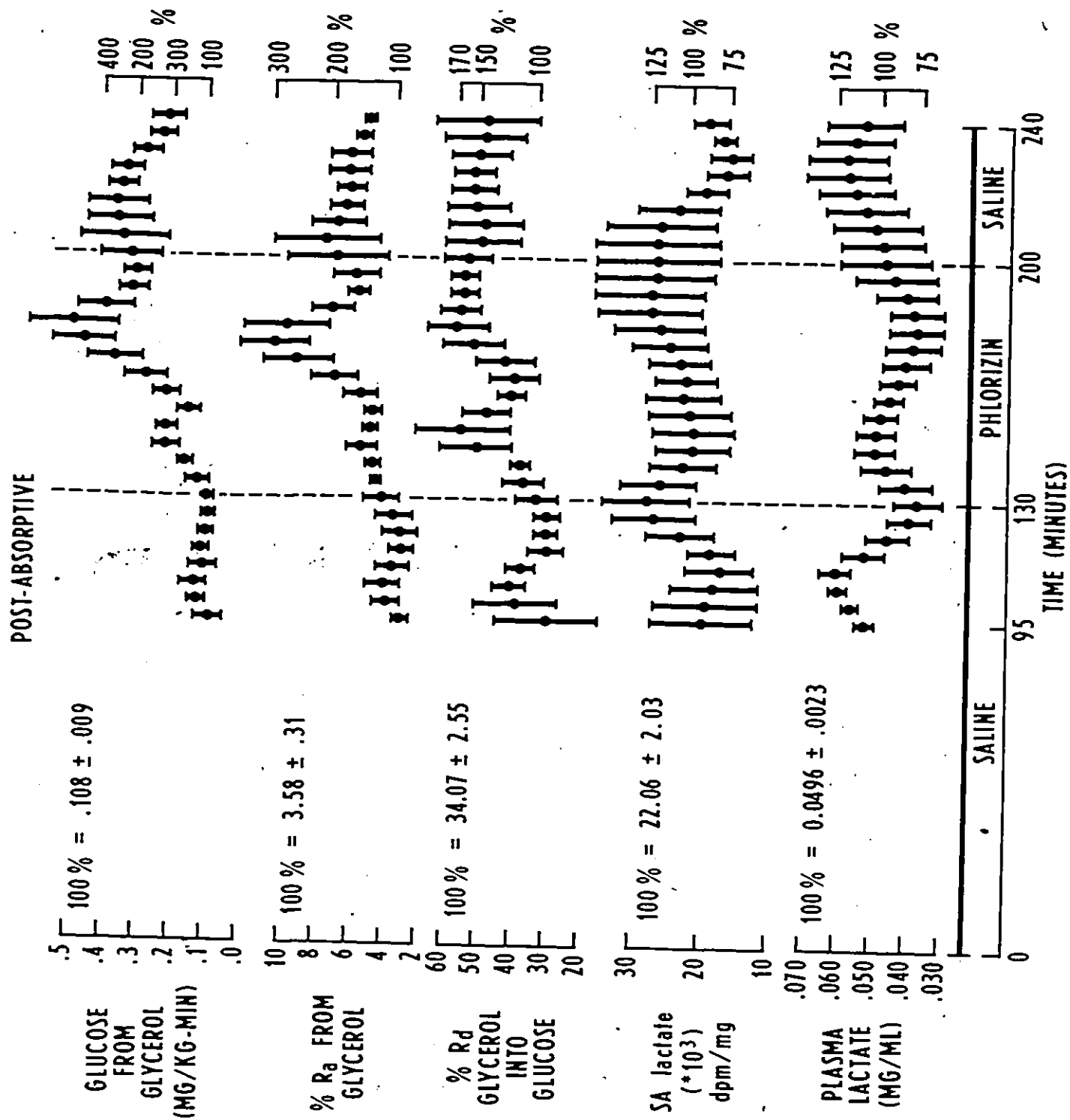
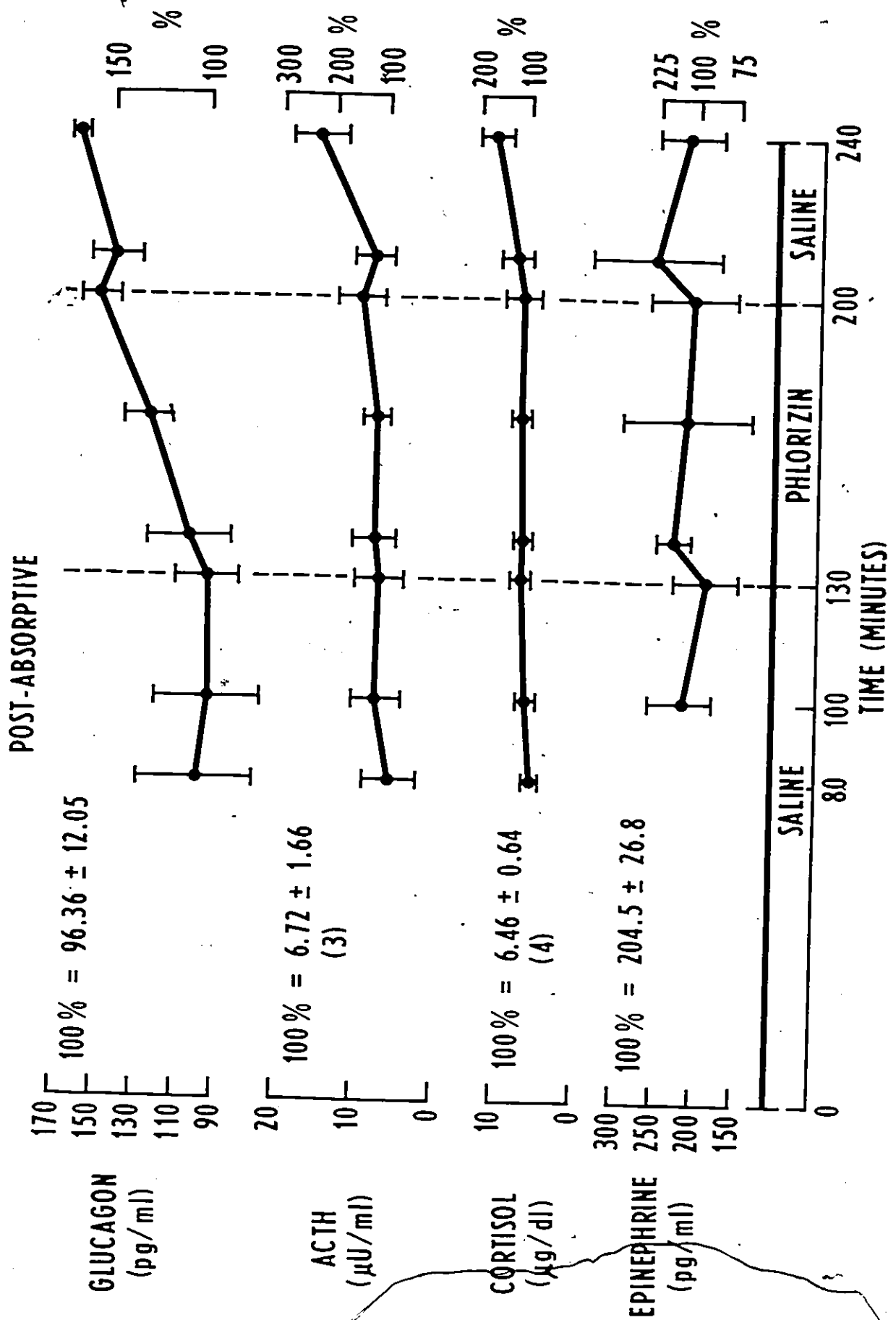


Figure 15. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the p.a. state, on plasma concentration of glucagon, adrenocorticotropin (ACTH), cortisol and epinephrine. Abscissa: time in minutes. Ordinates on the left: variables in absolute units. Ordinates on the right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. (n=4)



Figure 15



The fraction of glycerol turnover converted to glucose did not change significantly ($F = .10$); nor, was there any significant change in the specific activity and concentration of plasma lactate. The plasma concentrations of epinephrine, ACTH and cortisol did not change during the infusion of phlorizin (Fig. 15). After the cessation of the infusion of phlorizin the rate of gluconeogenesis from glycerol and the concentration of glucose in plasma began to return towards their basal levels, but no change in any other observed variable was noted during the recovery period of 40 minutes.

The results of experiments carried out on dogs fasted for 4 days are summarized in Fig. 16, 17 and 18. Under the near-steady-state conditions prevailing before the infusion of phlorizin, the plasma glucose and IRG levels were not significantly different from their respective values in the p.a. state, but the rate of glucose production was found to be significantly smaller ($t = 2.908$, $p < .05$). The plasma level and the rate of turnover of glycerol were somewhat higher than in the p.a. state ($t = 3.508$, $p < .05$, $t = 2.366$, $p < .05$). Glycerol was converted to glucose at about the same rate as in the p.a. state and accounted for the same percentage of glucose formation from glycerol ($t = 2.251$, $p < 0.10$). Despite the somewhat increased rate of glycerol turnover,

the fraction of glycerol converted to glucose was not significantly different from that observed in the p.a. state. Plasma lactate was not labelled to a higher extent than in the p.a. state. The ratio of the specific activity of glucose (SA-glucose) to the specific activity of lactate (SA-lactate) either decreased or did not change in individual dogs (Table 4). As shown in Fig. 18 neither the basal plasma glucagon, ACTH or epinephrine levels were found to be significantly different from values obtained in dogs in the p.a. state. The plasma cortisol level was found to be significantly higher ($t = 3.94$, $p < .05$).

During the infusion of phlorizin the concentration of IRG in the plasma and the rate of glucose production rose. This was also the case in dogs in the p.a. state, but the decrease in plasma glucose concentration was larger ($t = 4.365$, $p < .05$). Neither the concentration of plasma glycerol nor its turnover rate increased significantly. The increase in the rate of glucogenesis from glycerol however was significant ($F = 7.338$, $p < 0.05$), as was the increase in the fraction of glycerol turnover converted to glucose ($F = 6.637$, $p < 0.05$). However, the fraction of plasma glucose arising from glycerol remained essentially unchanged ($F = 3.439$, n.s.). The specific activity of lactate in the plasma increased ($F = 7.476$, $p < 0.05$) without any change in the concentration of plasma lactate ($F = 3.214$, n.s.).

The concentration of IRG ($F = 11.443$, $p < 0.01$), ACTH ($F = 20.408$, $p < 0.005$) and cortisol ($F = 4.729$, $p < 0.05$) in plasma all increased significantly, while the level of epinephrine did not change. After the cessation of the infusion of phlorizin only the concentration of ACTH and the SA of lactate showed a clear tendency to return to their pre-infusion basal level. The statistical analysis of the results of these experiments is given in Appendix IV and in Tables 42 to 75.

Figure 16. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the fasted state, on plasma glucose concentration, rate of appearance (Ra) of glucose, plasma glycerol concentration and rate of appearance (Ra) of glycerol. Abscissa: time in minutes. Ordinates on the left: variables in absolute units. Ordinates on the right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. (n=4)

Figure 16

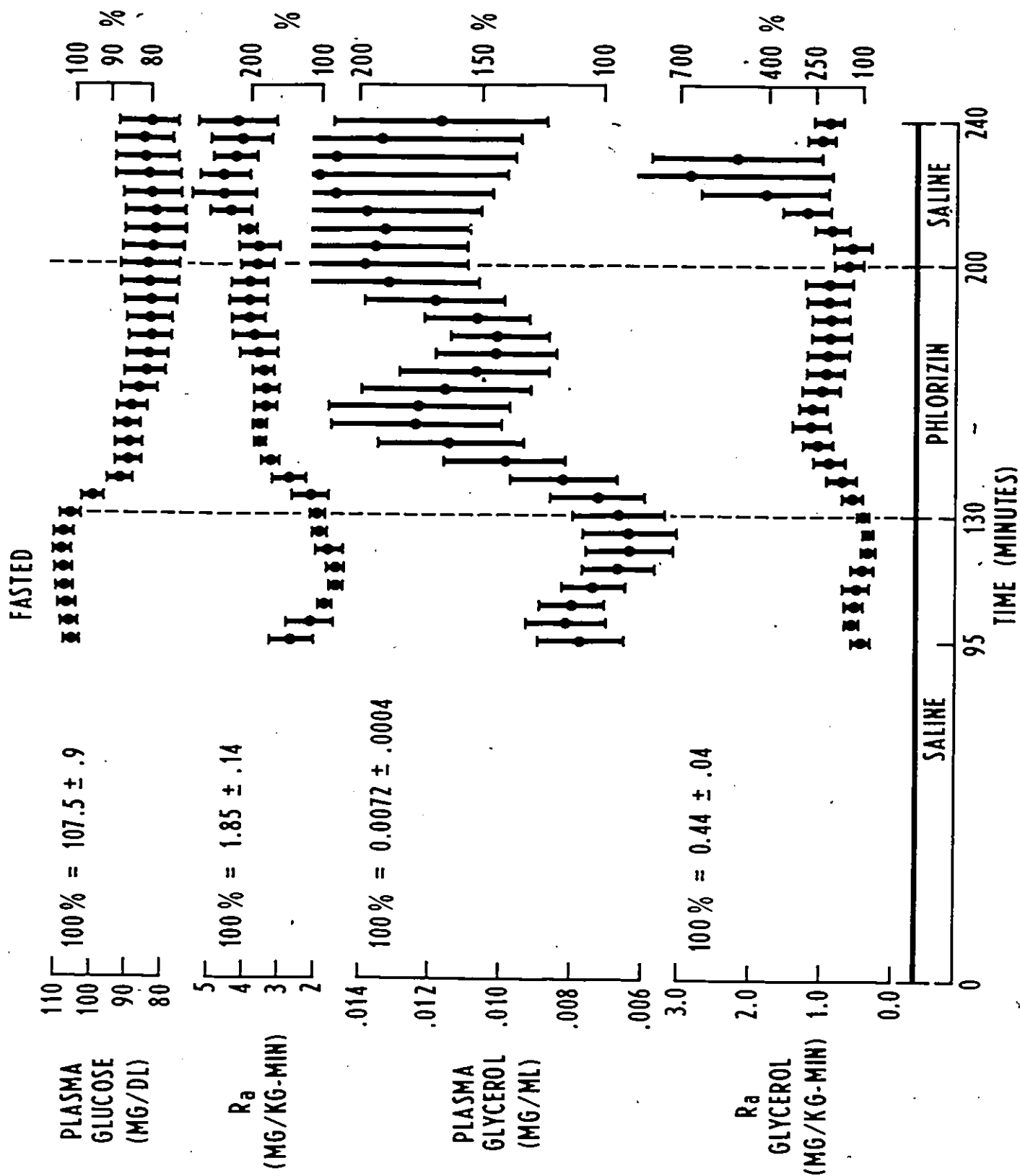


Figure 17. Effect of infusing phlorizin at $50 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in dogs in the fasted state, on the rate of formation of glucose from glycerol, the fraction of circulating glucose arising from glycerol, the fraction of utilized glycerol-C transferred to glucose, the specific activity of lactate and the plasma lactate concentration. Abscissa: time in minutes. Ordinates on the left: variables in absolute units. Ordinates on the right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. (n=4)

Figure 17

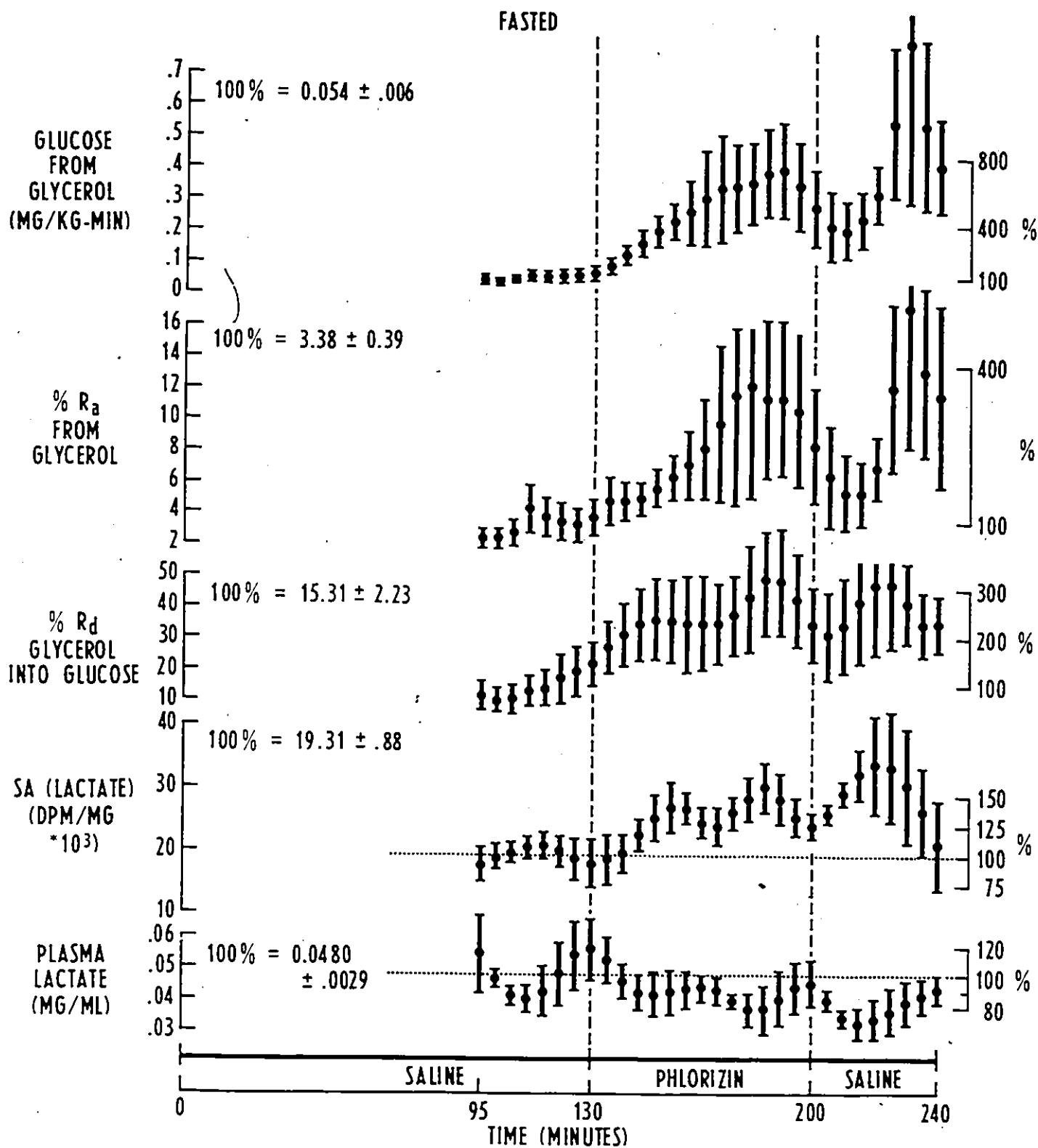


Figure 18. Effect of infusing phlorizin at $50 \mu\text{g.kg}^{-1}.\text{min}^{-1}$ in dogs in the fasted state, on plasma concentration of glucagon, adrenocorticotropin (ACTH), cortisol and epinephrine. Abscissa: time in minutes. Ordinates on the left: variables in absolute units. Ordinates on the right: variables expressed as percentage of their control values. Standard errors of means are shown as vertical bars. (n=4)

Figure 18

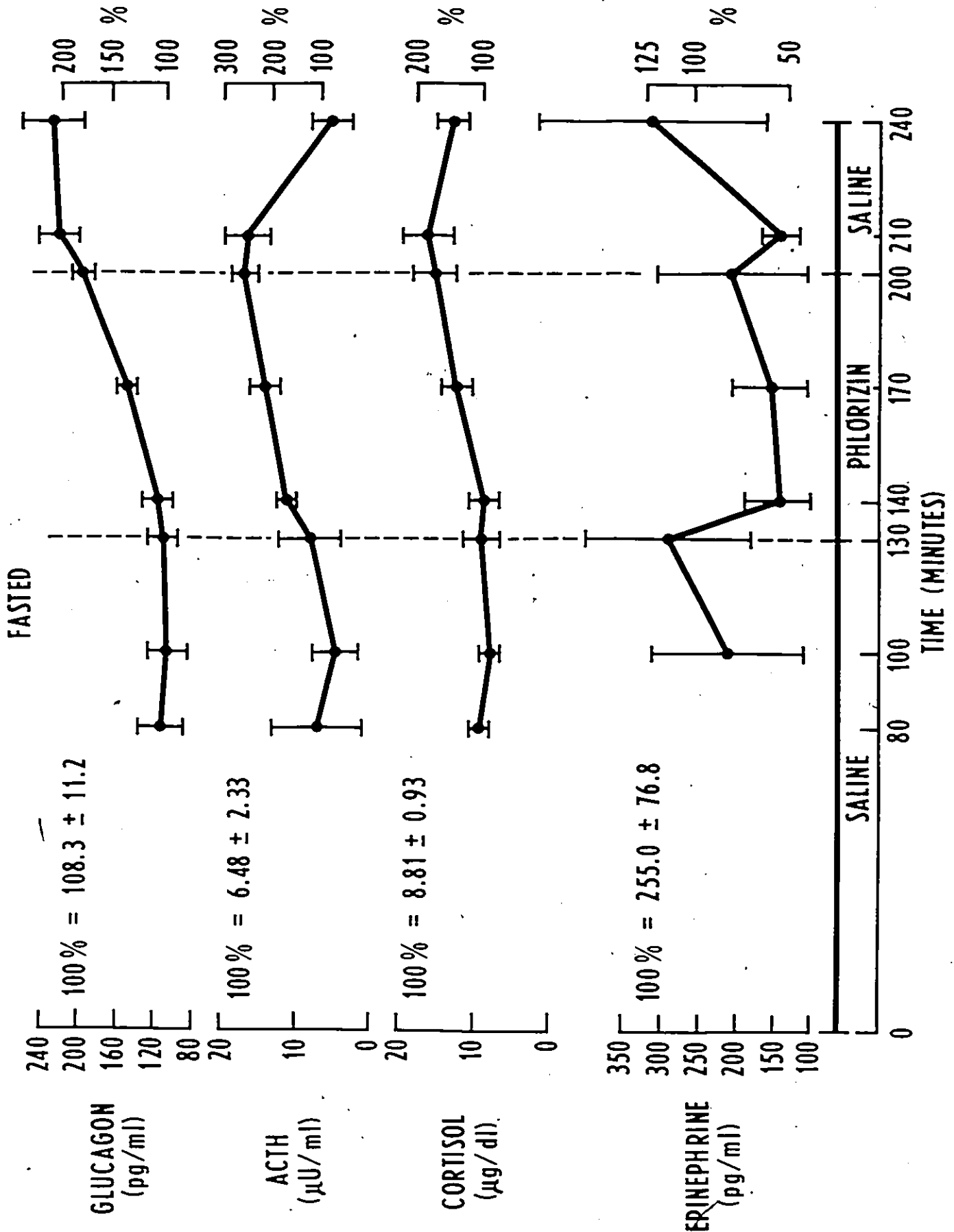


TABLE 4

The ratio of SA(glucose)/ SA(lactate) in the plasma of dogs in the postabsorptive (P.A.) state and after a 4 day fast.

Dog	p.a.	Fasted
1	0.21	0.15
2	0.22	0.10
3	0.62	0.77
4	0.52	0.62

TABLE 5

The effect of the infusion of phlorizin on the plasma alanine concentration ($\mu\text{mol/L}$) in conscious dogs in the postabsorptive and fasted (96 hrs) states.

TIME(min)

	SALINE					PHLORIZIN					SALINE				
	90	100	115	130	140	155	170	185	200	210	225	240			
POST- ABSORPTIVE	273 ± 65	288 ± 66	272 ± 78	272 ± 82	260 ± 78	280 ± 78	244 ± 79	278 ± 60	251 ± 77	236 ± 81	200 ± 65	192 ± 76			
FASTED	370 ± 79	335 ± 75	333 ± 76	310 ± 61	342 ± 71	299 ± 65	305 ± 63	307 ± 65	290 ± 45	267 ± 55	269 ± 63	261 ± 53			

Mean Values $\pm$ s.e.m. are shown

VI - DISCUSSION

First and Second Series: Gluconeogenesis and Glycogenolysis in Nonhypoglycemic and Hypoglycemic Glucoregulation.

a. The Sources of Plasma Glucose in the Postabsorptive and Fasted States.

In agreement with earlier findings (Cowan et al., 1969) a significantly lower rate of glucose production was found in fasted dogs than in dogs in the postabsorptive state. As a result of a significant decrease in the metabolic clearance rate of glucose, however, plasma glucose in fasted dogs fell only by about 6%. This finding in itself indicates the efficient long-term operation of the gluco-regulatory system, which is capable of maintaining a near "normal" level of plasma glucose, despite a substantial loss in stored carbohydrate. Fasting is characterised by a decrease in the secretion of insulin (Cahill et al., 1966) but, following a transient elevation, not consistently observed in the dog (De Bruijne et al. 1981), there is no apparent change in the level of glucagon in the plasma (Unger and Orce, 1976). The resulting change in the plasma insulin: glucagon ratio reflects the means by which a nearly normal level of glucose in the plasma is maintained in fasting (Lickley et al., 1979). The secretion of growth hormone

and the thyroid hormones do not seem to be affected by a short term of fasting. (De Bruijne et al., 1981).

During fasting, plasma glucose has to be synthesized by gluconeogenesis. Not all substrates in gluconeogenesis have been identified, but alanine and lactate appear to be among the most important quantitatively. The finding in the present study, of 7.2% of the C-atoms of plasma glucose arising from alanine is in agreement with earlier findings in dogs (Hall et al., 1977), when correction is made for the "metabolic exchange" (Hetenyi, 1979) of carbon atoms in the hepatic oxalo-acetate pool (Krebs et al., 1966). The quantitative interpretation of data on gluconeogenesis based on the transfer of ^{14}C -atoms from a selected substrate to glucose both present in the plasma is complicated by the interchange of C-atoms among the substrates, viz. between alanine and lactate. Rates of exchange among circulating lactate, alanine and glucose have been calculated by mathematical modelling (Foster et al., 1980). The combined contribution of C-atoms from lactate and alanine was found to account for 14% of those in glucose. If allowance for metabolic exchange is made (Hetenyi, 1979), the combined contribution of the two substrates is estimated to be about 30% of the glucose released by the liver, which is somewhat more than with our results.

In fasted dogs the basal contribution of alanine to plasma glucose was increased to 16%. About half of this increment was attributable to a decrease in the rate of glucose production, the other half to an increase in the rate of transfer of C-atoms from plasma alanine to glucose (compare Fig. 16 and 17). C-atom transfer was achieved without an increase in the rate of turnover of alanine. In man gluconeogenesis from alanine has been reported to double after 48hrs fasting (Chiasson et al., 1979).

b. The response to an Increased Removal of Glucose from the Circulation.

In response to an increase in the rate of disappearance of glucose from the circulation, induced by i.v. phlorizin, the regulatory increase in hepatic glucose production is at the expense of both glycogenolysis and gluconeogenesis in the postabsorptive as well as the fasted states. There are, however, differences between the two states in terms of the extent to which the rate of glucose release is raised and the time course of the response.

In the postabsorptive state, the rapid rise in glucose production during the infusion of phlorizin resulted from glycogenolysis. This is indicated clearly by this finding of synchronous rapid rises of both R_a and glycogenolysis

(Fig. 11), whereas the rise in gluconeogenesis peaked about 20 minutes later. This later peak was evident whether the rate of gluconeogenesis was estimated from the transfer of ^{14}C -atoms from alanine to glucose (Fig. 9) or by its recirculation from the first to the sixth position in plasma glucose (Fig. 11). Further evidence for the role of glycogenolysis is that in the fasted state the rise in R_a was smaller and somewhat delayed compared to that in the postabsorptive state, the concentration in plasma glucose decreased to a slightly lower level.

Increased gluconeogenesis does nevertheless contribute to the extra glucose released in response to phlorizin-induced glycosuria. The increase in gluconeogenesis is due to an increase in the fraction of the rate of disappearance of alanine which is turned to glucose. Such an effect of infused glucagon has been described previously (Chiasson et al., 1977). This increased gluconeogenesis is responsible for the sustained elevation of glucose production during the infusion of phlorizin, a finding in agreement with that of Cherrington et al., (1981), who reported that the effect of infused glucagon on glycogenolysis is transient, whereas that on gluconeogenesis is sustained. In their experiments on dogs, in which plasma insulin level was kept constant by a combined infusion of insulin and somatostatin,

the sudden elevation of the plasma glucagon level from an average of 55pg/ml to 206 pg/ml resulted in an initial 180% increase in glucose production, followed by a decline so that after 180 minutes glucose production was only 41% above basal. This latter increment appeared to be due entirely to an increased rate of glucose formation from alanine and lactate (Cherrington et al., 1981). It is noteworthy that in these experiments (Cherrington et al., 1981) the concentration of alanine tended to decrease after about 30 minutes of hyperglucagonemia. The trend was not reversed within 1 hour after having discontinued the infusion of glucagon. The data presented in the present study in Table 5 tend to support the earlier work. However, perhaps because of the smaller and less sudden increase in the concentration of plasma glucagon, the decrease was not significant statistically in our experiments.

Two conclusions may be drawn from these observations. First, glucagon released in response to an increased removal of glucose from the circulation (presumably a physiological stimulus), is capable of augmenting the rates of both glycogenolysis and gluconeogenesis. We interpret this as proof of both effects being "physiological", i.e. such as to be called upon in a regulatory, homeostatic process.

Second, the regulatory response (the increase in glucose production) does not critically depend on the availability of any single substrate or metabolic pathway. Different pathways all activated by glucagon can be and are called upon to achieve it.

The amount of glucagon released does nevertheless depend on the availability of ~~the readily~~ mobilizable substrate, hepatic glycogen. In the absence of glycogen the increase in hepatic glucose production is delayed, plasma glucose falls to a greater extent and consequently more glucagon will be secreted. The larger amount of glucagon will stimulate gluconeogenesis to a larger degree, and an imperfect but still adequate compensation for the loss of glucose will be achieved. In other words, in the absence of glycogen the regulatory system operates less efficiently, but still quite adequately.

If regulation against increased withdrawal of glucose from the circulation is largely independent of the availability of a single substrate, is it critically dependent on a single hormone, i.e. glucagon? The answer depends on the standards by which the effectiveness of the regulation is assessed. It has been shown that when the concentration of glucose in the plasma falls below 60-65 mg/dl catecholamines will be released in dogs (Goldfien et al., 1958), as well as in man (Garber et al., 1976).

These in turn stimulate hepatic glucose production and prevent the fall of plasma glucose below a critical level. (Altszuler et al., 1967). On the other hand, the "fine tuning" of glucoregulation within or slightly below its normal range seems to depend largely on glucagon, the effect of which may be modified by changes in the concentration of insulin in the plasma. Any small decrease in plasma insulin is expected to augment the sensitivity of the liver to the "extra" glucagon secreted in response to an increased rate of removal of glucose from the circulation. A small decrease in the concentration of plasma insulin has been observed occasionally during the infusion of phlorizin, but was not found to be statistically significant in either one of the two series of experiments reported by Gauthier et al., (1980). Re-analysis of the data (Gauthier et al., 1980) revealed that whereas the concentration of glucagon in plasma has increased during a 70 minute infusion of phlorizin by a factor of 2.3, the "glucagon: insulin ratio" increased by a factor of 2.5 in one and 4 in the other series of experiments. Thus the marginal decrease in plasma insulin level might have made glucagon secreted more effective.

c. The Response to Insulin-Induced Hypoglycemia.

In two dogs in the p.a. state the level of plasma glucose was decreased to 45-50 mg/dl by the infusion of insulin. The rates of glucose production were observed before, during and after the infusion. The changes in the rates in glycogenolysis and gluconeogenesis were also monitored as described in Materials and Methods, Calculations (b.2.1. and b.2.2.). As it can be seen in Fig. 12 the glycogenolytic index used was validated by the steady concentration of ^3H -6-glucose in the plasma prior to, and its sustained increment after the infusion of phlorizin was terminated during which the concentration of plasma glucose remained below basal levels. At this low level of glycemia, not only the secretion of glucagon, but also of catecholamines, adrenal corticoids and growth hormone are elevated (Goldfien et al., 1958; Garger et al., 1976). Therefore the comparison of the effects of insulin-induced hypoglycemia and of the compensated loss of glucose from the circulation caused by phlorizin permits the assessment of the role of other hormones in glucoregulation, particularly since the increments in the concentration of plasma glucagon are comparable. In overt hypoglycemia the rate of glucose production was trebled when the concentration of glucose in the plasma leveled off at 45 mg%. Again both glycogenolysis and gluconeogenesis increased, but the lack of

a sustained effect in the former seemed to be responsible for a slight decline in R_a after about 40-50 minutes.

Glycogenolysis was increased much more than during the infusion of phlorizin. This indicates a significant effect of catecholamines on glycogenolysis in hypoglycemia. The large increase in gluconeogenesis was achieved in spite of the well known inhibitory effect of insulin. In view of the effect of adrenaline on the Cori-cycle (Altszuler et al., 1967) the enhanced incorporation of ^{14}C -atoms into the 6th C-atom of plasma glucose is likely due to the faster turnover of the Cori and the alanine-glucose cycles. Evidence for a simultaneous increase in both glycogenolysis and gluconeogenesis during insulin-induced hypoglycemia has been presented earlier by Cherrington et al. (1979).

As already mentioned, these experiments had to be analyzed on the basis of the two-compartmented model of the glucose system (Radziuk et al., 1978) because the generally used single compartmental model led to contradictory results in the calculation of the changes in the rate of glycogenolysis. However, this is hardly surprising in view of the rapid and large deviations from dynamic steady state, when the assumption of a single well mixed compartment of a definite and constant size becomes untenable. By comparison, in the experiments in which phlorizin was infused, the deviations from steady

state were small and R_a calculated by either the single compartmental approximation of Steele et al. (De Bodo et al., 1963), or by the assumption of the two compartmented model of Radziuk et al. (Radziuk et al., 1978) were essentially identical. (Table 3).

Third Series: The Role of Glycerol and Epinephrine, ACTH and Cortisol in Nonhypoglycemic Glucoregulation.

a. Glycerol and Glucose Metabolism in the Postabsorptive State and after Four Days Fasting.

In the postabsorptive state the mean turnover rate of glycerol found in the experiments (0.34 ± 0.03 mg/kg.min) is in agreement with those found by other investigators (Hall et al., 1976; Shaw et al., 1975; Winkler et al., 1970). Thirty four percent of the C-atoms of glycerol disappearing from the circulation were converted to plasma glucose, the same percentage as calculated by Winkler et al. (1970). Hall et al. (1976) using a different experimental approach, found that 48% of C-atoms were converted. In lean men Bortz et al. (1972) found 38% of the carbon atoms of plasma glycerol transferred to plasma glucose. We found a considerable fraction of the label infused as glycerol in plasma lactate. Indeed, in a steady state, the ratio of SA(lactate)/SA(glucose) during the infusion of ^{14}C -glycerol was much higher (3.2 ± 0.8) than 0.32 ± 0.035 , the SA(lactate)/SA(glucose) ratio found by Forbath and Hetenyi (1970) during the infusion of ^{14}C -glucose in dogs. It has to be remembered that the SA of both lactate and glucose measured in mixed venous blood is lower than that of lactate and glucose produced by gluconeogenesis. The former because of a dilution by peripherally produced lactate, the latter because of dilution by glucose originating by glycogenolysis. Our findings nevertheless indicate that a sizable fraction of glycerol that enters glycolysis travels "downstream" towards lactate.

2

Since the sole precursor of lactate is pyruvate, which at the same time is a main fuel of the Krebs cycle, it may be suggested that a larger fraction of glycerol appears to be oxidized than turned to glucose. This is also evident from the fraction (66%) unaccounted for by glucose formation. In this study, the rate at which glycerol was converted to glucose accounted for 3.6% of hepatic glucose production, somewhat less than 6% calculated by Winkler et al. (1970) or 7% as given by Steele et al (1971), both using a tracer technique in many respects similar to the one used in our experiments and to the 6.1% calculated by Hall et al. (1976) by a different approach. In man, Bortz et al. (1972) found a contribution of 3%.

A four day fast did not change significantly the levels of plasma glucose and IRG. In dogs, De Bruijne et al. (1981) found no significant changes in either of these variables, nor in the level of glycerol before the 5th day of starvation. Indeed the level of plasma glucagon did not change at all even after 14 days of food deprivation. A small but significant increase in the plasma level and turnover after longer starvation were found by Hall et al. (1976). The fraction of glycerol turnover converted to glucose decreased from 34% to 15% after fasting. A smaller but significant decrease was observed also by Hall et al. (1976)

in dogs fasted for 7 days. It appears that during fasting of 4-7 days duration glycerol is used preferentially as fuel for oxidation rather than as a substrate for gluconeogenesis. It is of interest that the basal SA(glucose)/SA(lactate) ratio in dogs 1 and 2, which were less than half of that observed in dogs 3 and 4 in the postabsorptive state, decreased even further after 4 days of fasting, whereas no such decrease was evident in dogs 3 and 4 (Table 4). An explanation for this difference is not obvious at this time.

b. The Conversion of Glycerol to Glucose and Lactate.

Carbon atoms (including ^{14}C -atoms) from glycerol enter the glycolytic pathway and contribute both to the formation of lactate by travelling "downstream" and to glucose by travelling "upstream". The present finding that plasma lactate is more heavily labelled during the infusion of glycerol when ^{14}C -U-glucose is infused indicates the importance of the downstream pathway.

The ratio of SA (lactate)/SA(glycerol) in plasma was 0.091 ± 0.027 in the postabsorptive state and 0.107 ± 0.024 in fasted dogs. Thus about 9-11% of the C-atoms in plasma lactate seem to originate from glycerol. The true fraction may be higher, however, because the C-atoms in the pyruvate pool in muscles almost certainly undergo metabolic exchange with CO_2 and other metabolites.

The SA(glucose)/SA(lactate) ratio in the postabsorptive steady state was found to be 0.39 ± 0.10 during the infusion of labelled glycerol (Table 4). During the infusion of ^{14}C -U-lactate Forbath and Hetenyi (1970) reported this ratio to be equal to 0.23 ± 0.04 . Thus it appears that about 60% (i.e. $0.23/0.39$) of the label in plasma glucose during the infusion of ^{14}C -glycerol can be accounted for by the transfer of ^{14}C -atoms from lactate to glucose. Indeed even 100% is not outside the fiducial 5% limits of the calculated ratio. Since no data on the ratio is available on fasted dogs during the infusion of ^{14}C -U-lactate this calculation cannot be carried out in this nutritional state. The observed value of 0.41 ± 0.17 suggests the possibility that a large fraction of the label in plasma glucose can be accounted for by labelling lactate.

c. Glycerol as a Source of Glucose Released During the Infusion of Phlorizin

In earlier studies (Gauthier et al, 1980), it was found that when the rate of removal of glucose from the circulation is increased by phlorizin-induced glycosuria, the rate of hepatic glucose production increases. The increase is mainly due to an increment in the release of glucagon. When the secretion of glucagon is blocked by somatostatin no increment is found in plasma IRG concentration and the concentration of plasma glucose falls.

The sources of the extra glucose mobilized by glucagon were both from glycogenolysis and gluconeogenesis from alanine, the latter due to a more efficient conversion of alanine to glucose and not the mobilization of alanine (Gauthier et al., in press). All these observations can be accounted for by the release of glucagon (Liljenquist et al., 1979).

In contrast to alanine, glycerol is a by-product of lipolysis, a process on which catecholamines and to a minor extent ACTH but not glucagon have effects. Should the formation of glucose from glycerol be determined solely by the mass action of available glycerol, it would be expected that the fraction of glycerol turnover converted to glucose would remain the same during and before the infusion of phlorizin. This was indeed the case in the postabsorptive state, but not in fasted dogs.

In dogs in the fasted state a significant increase in the fraction of glycerol turnover converted to glucose was observed during the infusion of phlorizin. This indicates that increased gluconeogenesis from glycerol was not due to an increased mobilization of glycerol, but instead to a re-distribution of the C-atoms in glycerol among different metabolic pathways. In fasted dogs the infusion of phlorizin lead to a 50% increase in SA of plasma lactate without any

change in the plasma lactate level, This increment might have accounted for the increased transfer of ^{14}C -atoms from glycerol to glucose. It should be noted that in the post-absorptive state no increase in the SA(lactate) in plasma and no increase in the calculated fraction of glycerol-C to plasma glucose was evident. It is of further interest that the level of ACTH and cortisol were raised in the fasted but not in the postabsorptive state during the infusion of phlorizin and the plasma concentration of ACTH and the SA(lactate) are the only two measured variables which appear to return near to their basal levels within 40 minutes after the cessation of the phlorizin infusion. The underlying cause of this correlation however remains obscure.

Since in fasted dogs, no significant increase in the fraction of circulating glucose originating from glycerol was observed during the infusion of phlorizin, glycerol does not seem to be an important precursor for the synthesis of glucose, but rather appears to be a major fuel which can be channelled rapidly to Krebs cycle, thus sparing glucose. ACTH and/or cortisol are potential agents effecting this rapid "downstream channelling" of glycerol in response to the infusion of phlorizin, although the mechanism of their action remains unknown.

d. The Release of Epinephrine, ACTH and Cortisol.

In the postabsorptive state the plasma levels of these hormones was found not to be increased during phlorizin-induced glycosuria. Thus whatever their effects on lypolysis or hepatic glucose production may be, they do not mediate the rapid response to an increased loss of glucose from the circulation. The lack of a significant increase in the concentration of plasma epinephrine is in accordance with our earlier finding of no change in plasma free fatty acids level during phlorizin-induced glycosuria (Gauthier et al., 1980)

In fasted dogs, the basal (pre-infusion) level of plasma epinephrine was not significantly different from that found in the same dogs in the postabsorptive state (t 1.415, n.s.). The levels of nor-epinephrine and dopamine remained unchanged as well. The small increment in plasma cortisol in fasted dogs was of marginal significance (t 2.35, p .05).

As in the postabsorptive state, again no significant change in plasma epinephrine concentration was found during the infusion of phlorizin in fasted dogs, although the level of plasma glucose fell to a somewhat lower level than in the experiments in the postabsorptive state. The significant increase in the plasma concentration of ACTH and cortisol may be accounted for by this larger decrement in plasma glucose

concentration and perhaps also by the stress effect of fasting which may have increased the sensitivity of the pituitary-adrenal axis to other stimuli.

The results lead to the conclusion that regulatory compensation of mildly hypoglycemic stimuli is primarily mediated by secretion of extra glucagon, the effect of which may be augmented by a marginal decrease in the secretion of insulin. Epinephrine, ACTH and cortisol have no appreciable role in glucoregulation near the euglycemic level.

In overt hypoglycemia when the concentration of glucose in the plasma is below 60 mg/dl the secretions of catecholamines, ACTH and cortisol are stimulated. However, even under such conditions only the former but not the latter two have any impact on hepatic glucose release and thus on glucoregulation. In man extra cortisol and growth hormone are released into the circulation in response to insulin-induced hypoglycemia but have no appreciable effect on the speed of the restoration of normal plasma glucose level (Gerich et al., 1980). It appears that the pituitary response to hypoglycemia (i.e. the secretion of ACTH and GH) is a response to the stress created by neuroglycopenia rather than a true glucoregulatory response. Indeed the slowly developing effects of these hormones on gluconeogenesis put them outside the time frame expected from effective regulatory agents (Gerich et al., 1980).

In man either adrenalectomy or adrenergic blockade have little, if any effect on recovery from hypoglycemia, but when superimposed on glucagon deficiency causes a further impairment in the restoration of normoglycemia compared to that observed in isolated glucagon deficiency (Gerich et al., 1980). In both man and dog however catecholamines are secreted only in deep hypoglycemia and their likely function is to augment the effect of glucagon or replace it should the secretion of glucagon be impaired for any reason.

In summary, the results of our experiments reveal that: (1) In dogs in the postabsorptive state, glucagon released in response to an increased removal of glucose from plasma increases the rate of glucose production by stimulating both gluconeogenesis from alanine and breakdown of hepatic glycogen, glycerol contributing only to a minor extent to the increment. (2) In fasted dogs in which the basal rate of gluconeogenesis was twice as high as in dogs in the p.a. state, the infusion of phlorizin caused a further increase. The increase was achieved by a larger fraction of utilized alanine being converted to plasma glucose. In these dogs, glycerol is a substrate more of oxidation than gluconeogenesis. (3) During the infusion of phlorizin, extra epinephrine is not released together with glucagon in either the postabsorptive or fasted state.

ACTH and cortisol are released only in dogs in the fasted state, the release of ACTH being closely correlated with an increase in the specific activity of lactate.

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VIII- APPENDIX

APPENDIX I

CONSTRUCTION AND IMPLANTATION OF A
PERMANENT CANNULA

a. Cannula Construction

As shown on Fig 19 , Anthony's cannula consists of 2 polyethylene tubings (Clay-Adams, New Jersey.U.S.A.): a PE-60 (i.d. 0.76 mm-o.d. 1.22 mm) and a PE-205 (i.d. 1.57 mm-o.d. 2.08 mm). The PE-60 must be shorter than the PE-205 by 0.5-1.0 cm so that once the whole cannula is assembled (Fig 19) the feather edge tips of the cannulae are correctly positioned. 1.5 cm stainless soldered plugs (16 and 21 G.A.) were prepared to fit the exposed end of the cannulae.

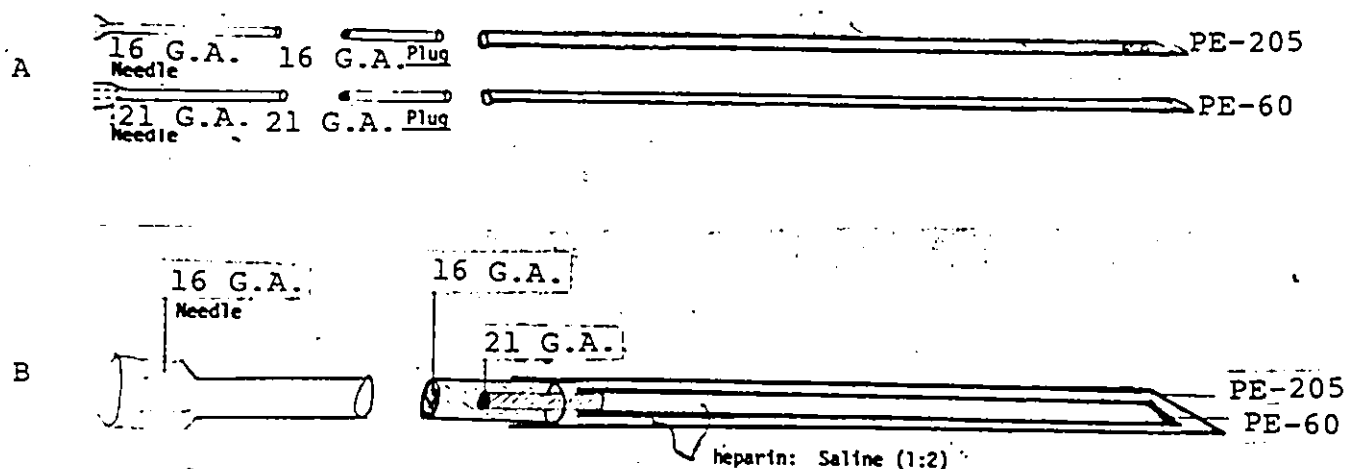


Figure 19 . Anthony's Cannula: A.disassembled
B.assembled

b. Cannulation and Implantation

Since the saphenous vein was chosen for cannulation, the distance between the exposure site (lateral aspect of the tarsus) and the sternum was estimated prior to cannulation, allowing an extra 3 cm coming out of the vein; the cannula was prepared accordingly.

After exposure of the vein under nembutal anaesthesia, an incision was made and the PE-205 cannula was inserted and pushed into the inferior vena cava. It was then pulled back for 2-3 cm. The incision was closed with surgical silk and the ^3H -6-glucose infused according to Radziuk and Vranic (1977) for 4 hours.

Skin sutures were removed and the PE-205 cannula was flushed with 0.9% NaCl. The PE-60 cannula was filled with heparin (1000 USP unit/ml): saline (1:2) and plugged with the 21 G.A. plug prior to its insertion into the PE-205. 0.5-0.7 ml heparin:saline (1:2) was gently pushed through the PE-205 cannula using a 16 G.A. needle, in order to fill the inter-cannular space. The PE-205 was then clamped and plugged with the 16 G.A. Finally, the exposed portion of the Anthony's cannula was tucked under the skin and the incision was carefully closed with surgical silk. Dogs were given 50,000 units penicillin (Penlong S, Rogar/STB) i.m.

On the day of the experiment, the skin sutures were removed, the PE-205 cannula unplugged and the PE-60 gently pulled out of the PE-205. 1.5-2.0 ml of blood was withdrawn to get rid of remaining heparin in the cannula which was subsequently flushed with saline, and ready to be used.

Anthony's cannula was useful in the experiments for two reasons: (1) it offered an efficient physico-chemical block to coagulation, allowing repeated use of the same dog without need for further cannulation and (2) a minimal amount of heparin reached the circulatory system, minimizing the risk of heparin-induced lipolysis, which could have interfered with gluconeogenic measurements.

Anthony's cannula has been tested for the first time on our 5 dogs up to 3 days after implantation. It has been shown to be effective till 8 weeks after implantation thereafter in dogs (Cowan, personal communication) and in pigs (Kucharczyk, personal communication) without deleterious secondary effects.

APPENDIX II

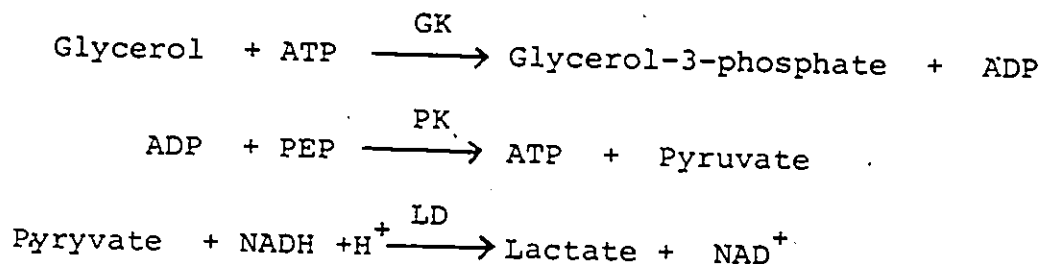
DETERMINATION OF THE CONCENTRATION AND SPECIFIC ACTIVITY
OF PLASMA GLYCEROL IN A SINGLE ASSAY

ABSTRACT

The determination of the concentration and the specific activity of plasma glycerol in a single assay system is described. The determination of the concentration of glycerol is based on its quantitative conversion to glycerol-3-phosphate (G3P). The resultant G3P is recovered and after the removal of anionic metabolites (e.g. lactate) is isolated on, and eluted from a Sephadex column and counted in a liquid scintillation spectrometer. The specific activity of glycerol so calculated was $100 \pm 3\%$ of a standard of known specific activity.

INTRODUCTION

As an alternative to the enzymic methods for the assay of glycerol based on the reduction of NAD to NADH_2 (Himms-Hagen, 1962; Wieland, 1957), Garland and Randle (1962) developed a more rapid method based on the coupling of the rapid and complete phosphorylation of glycerol to glycerol-3-phosphate (G3P) with the oxidation of NADH_2 according to the following reactions:



In experiments where ^{14}C -U-glycerol is used as the tracer to calculate the turnover of glycerol, it is necessary to isolate ^{14}C -U-glycerol from the plasma. The isolation of glycerol as glycerol-tribenzoate (Winkler et al., 1967) has been reported to yield falsely high specific activities due to the incomplete separation of plasma glycerol- ^{14}C from the ^{14}C -atoms in glucose (Bergman et al., 1968) and lactate (Gauthier et al. in preparation). Bortz et al (1972) have removed lactate- ^{14}C and some other negatively charged (anionic) metabolites by passing the filtrate through an IRA-410 resin column and precipitated the glucose- ^{14}C with CuSO_4 and $\text{Ca}(\text{OH})_2$. The isolation of ^{14}C -glycerol by paper chromatography (DeFreitas, 1967) followed by combustion and the trapping of $^{14}\text{CO}_2$ in ethanolamine (Hall et al., 1976) yielded a recovery of 88.5 ± 1.2%. Sephadex column chromatography has been used to achieve a complete separation of ^{14}C -G3P from free ^{14}C -glycerol in the radiochemical assay for unlabelled glycerol (Barrera and Ho, 1978).

The method to be described uses the procedure of Garland and Randle (1962) for the rapid and complete phosphorylation of ^{14}C -glycerol to ^{14}C -G3P. The latter is subsequently isolated by ion-exchange column chromatography.

MATERIALS AND METHODS

The enzymic assay of glycerol:

The concentration of glycerol was determined enzymically with a Glycerol UV kit purchased from Boehringer Mannheim Corp. The assay system was modified to contain 0.35 ml buffer, 0.55 ml distilled water, 0.01 ml pyruvate kinase-lactate dehydrogenase suspension, 0.01 ml glycerokinase suspension and 0.10 ml sample. The completeness of the conversion of glycerol to G3P was assessed on a Unicam S.P. 800 Ultraviolet Spectrophotometer by continuous reading for 10 minutes after the addition of the enzymes. The assay was tested in the range of 5 - 500 μM glycerol. The assays were carried out in triplicates except at the levels of 5 and 10 μM , for which 5 and 6 replicates were processed.

Isolation of ^{14}C -glycerol and determination of the specific activity of glycerol in untreated samples:

After the enzymic assay of a standard glycerol solution containing ^{14}C -U-glycerol 4.86×10^5 dpm/ml and 50 μM unlabelled glycerol was completed with 9 replicates, the product, ^{14}C -G3P, was isolated by the following procedure:

The contents of the spectrophotometric cuvette were transferred to a 5.0 ml glass tube and the cuvette was rinsed twice with 1.0 ml distilled water. The rinsings were added to the same tube. 0.10 ml carrier L- α -glycerophosphate (3.6 mM in ethanol) was added and the volume was made up to 4.0 ml with distilled water. The tube was covered with parafilm and inverted a few times. The contents of the tube were transferred to a QAE Sephadex column (0.7 x 3.5 cm) which was previously equilibrated (2 hours boiling) with 100 mM Tris-HCl buffer, pH 8.3. After the contents of the tube were put through the column, the tube was rinsed with 3.0 ml distilled water which was added to the column. Finally a further 3.0 ml distilled water was added to the column directly. ^{14}C -G3P was eluted with 10.0 ml of 0.1 N HCl. Two ml of this eluate was pipetted into a glass counting vial, 17.0 ml scintillation fluid (ScintiVerse) was added, and radioactivity was estimated in a Nuclear Chicago liquid scintillation counter (model 8912A). The specific activity (SA_0) was calculated as the ratio of the total ^{14}C -glycerol activity (G^*_0) expressed as disintegrations per minute (dpm) per ml to the concentration of the glycerol standard (G_0) in the cuvette, expressed in nmoles per ml, for each of the nine replicates.

Adsorption of ^{14}C -glycerol, ^{14}C -glucose and ^{14}C -lactate by the Sephadex column:

Solutions containing 5×10^4 dpm of ^{14}C -Uglycerol 50 μM or 5×10^4 dpm ^{14}C -U-glucose 5.5 mM or 4.4×10^4 dpm of ^{14}C -U-lactate 0.56 mM were passed through Sephadex columns as described for the isolation of ^{14}C -G3P. Five replicates were done for each solution.

Isolation of ^{14}C -glycerol and determination of the specific activity of glycerol in purified samples:

After passing 0.5 ml of the solution containing ^{14}C -U-glycerol 4.86×10^5 dpm/ml and 50 μM glycerol to ionic exchange resins (Reilly 1975a:Reilly 1975b) and subsequent deproteinization of the eluate with $\text{Ba}(\text{OH})_2$ and ZnSO_4 : 3.0 ml of the 1:16 filtrate were transferred to a siliconized vial and freeze-dried in a Labconco Freeze Dry-5. The contents of the vials were redissolved in 0.70 ml distilled water, of which 0.65 ml was used for the determination of glycerol concentration and the isolation of ^{14}C -glycerol, replacing the 0.55 ml distilled water and the 0.10 ml sample in the procedure described. Nine replicates were done. The specific activity (SA_1) was calculated by the method already described for SA_0 , that is, as the ratio of the ^{14}C -glycerol activity (G^*_1)

to the glycerol concentration (G_1) in the cuvette. An alternative method was also used for the calculation of the specific activity (SA_2) according to the following equation:

$$SA_2 = \frac{G^*_1 \times 1.1}{G_0 \times 1.1741} = \frac{G^*_2}{G_2}$$

where G^*_2 is the ^{14}C -glycerol activity G^*_1 corrected for partial loss during the removal of contaminants (see Table I), i.e. $100/90.9$, and G_2 is G_0 corrected for the dilution of the sample, i.e. $(3/16 \times 0.65/0.70)/0.10$.

Statistical analysis

The differences between SA_1 and SA_2 , and the expected specific activities (SA_0) as well as the differences between SA_1 and SA_2 were compared. A complete analysis of variance (Snedecor, 1956) was carried out on the specific activity data. The F and P values calculated for the differences were taken as measure of significance.

Chemicals:

^{14}C -U-glycerol (lot number 1060-239, 152 mCi/mol), ^{14}C -U-glucose and ^{14}C -U-lactate were obtained from New

New England Nuclear Corp., Boston, U.S.A.. The initial radiochemical purity of the tracers was greater than 99%. Amberlite resin IRA-400(Cl) 100-200 mesh and Amberlite resin IR-4B(OH) were obtained from BDH Laboratories, Philadelphia, U.S.A.. QAE Sephadex (stock no. Q-25-120) and L- α -glycerophosphate were obtained from Sigma Chemical Co., St-Louis, U.S.A. All chemicals were of the highest grade of purity available.

RESULTS AND DISCUSSION

Range of the enzymic assay of glycerol:

The measured mean value, as calculated from the molar extinction of NADH_2 , and the theoretical value for each standard glycerol concentration are shown on Fig 20. As it can be seen, the enzymic assay departs significantly from linearity below 10 μM and beyond 250 μM . However, over a range of glycerol concentrations of 10 - 250 μM , change of optical density at 340 $\text{m}\mu$ and the concentration of glycerol are linearly related.

Isolation of ^{14}C -glycerol and determination of the specific activity of glycerol in untreated samples:

As evident from Table 5, the method described is very efficient and precise in isolating ^{14}C -glycerol as its phosphorylated derivative, since $96.6 \pm 0.2\%$ of ^{14}C -glycerol is recovered as ^{14}C -G3P. It is important to note that the Sephadex column adsorbs practically all glycerophosphate, since the enzymic assay revealed the concentration of standard glycerol to be only $97.1 \pm 2.7\%$ of the nominal $50 \mu\text{M}$. Accordingly $100.1 \pm 2.9\%$ of the expected specific activity was found.

Adsorption of ^{14}C -glycerol, ^{14}C -glucose and ^{14}C -lactate by the Sephadex column:

It was found that $0.7 \pm 0.1\%$ of ^{14}C -glycerol and about the same amount of ^{14}C -glucose were eluted with 10.0 ml of 0.1N HCl , while $88.6 \pm 0.6\%$ of the ^{14}C -lactate was retained by the column and only $5.5 \pm 0.6\%$ was eluted with the first 10.0 ml of distilled water, compared to $98.6 \pm 0.4\%$ and $100.2 \pm 0.9\%$ for ^{14}C -glycerol and ^{14}C -glucose respectively. Significant contamination of ^{14}C -G3P by ^{14}C -glycerol or ^{14}C -glucose may therefore be excluded. However, the potential contamination by labelled anions such as lactate and anionic compounds known to exchange ^{14}C -atoms with lactate, such as

alanine, has to be prevented. These contribute to the contamination observed by Bortz (1972). Negatively charged contaminants can be removed from the sample prior to the enzymic assay as described above. Since the Sephadex column has been shown not to retain uncharged labelled molecules and the filtrate passed through the ionic-exchange resins is nearly free from ionic labelled contaminants, the ^{14}C -G3P arising from the phosphorylation of ^{14}C -glycerol will be the only ^{14}C -labelled anion trapped by the Sephadex column and released by 0.1 N HCl.

Isolation of ^{14}C -glycerol and determination of the specific activity of glycerol in purified samples:

As shown on Table 5.1 in the purified sample, the mean concentration of glycerol and the ^{14}C -glycerol activity in the cuvette are 7.59 ± 0.44 nmole/ml and 74631 ± 575 dpm/ml. This represents $90.0 \pm 0.7\%$ of the expected values, which means that about 9% was lost in the various steps of purification. The specific activities, SA_1 and SA_2 , as calculated by the two methods described above are also shown. No statistically significant differences were observed between the expected specific activity, SA_0 , and the specific activity of the purified sample calculated by either of the two methods, SA_1 and SA_2 .

The method described is a very efficient tool for the study of glycerol metabolism since: (1) the range of the enzymic assay is suitable for studies in animals undertaken under various nutritional states; (2) the method allows the isolation of pure ^{14}C -glycerol with a yield of $96.6 \pm 0.2\%$; (3) it allows the calculation of the specific activity of glycerol in the same assay system, that is in the spectrophotometric cuvette. The volume, or the concentration of glycerol in the sample, is not a limiting factor, and various adaptations of the method may be considered, such as the alkaline hydrolysis of triglycerides (Eggstein and Kuhlmann, 1974) prior to the enzymic assay of glycerol, which would permit the calculation of the rates of exchanges between triglycerides and glycerol pools.

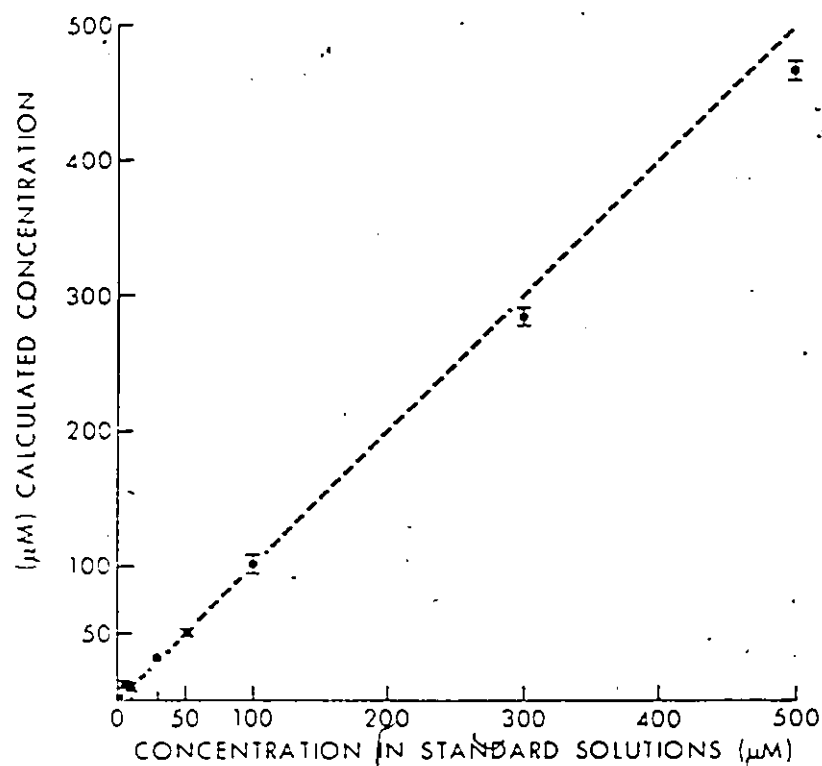


Fig 20 Determination of the concentration of glycerol. Concentrations calculated from the formation of NAD (vertical co-ordinate) are plotted against standards (horizontal co-ordinate). Mean values $\pm$ S.D. are shown.

Table 5. ^{14}C -glycerol activity (G^*), glycerol concentration (G) and specific activity (SA) in the cuvette for untreated and purified samples calculated by methods 1 and 2 (see text).

	G^* (dpm/ml)	G (nmole/ml)	SA (dpm/nmole)
Without pre-treatment	47155 ± 74 (96.6 $\pm$ 0.2%)	4.74 $\pm .13$ (97.1 $\pm$ 2.7%)	10010 ± 288 (100.1 $\pm$ 2.9%)
With purification:			
Method 1	74631 ± 575 (90.0 $\pm$ 0.7%)	7.59 $\pm .44$ (92.0 $\pm$ 5.3%)	10143 ± 612 (101.3 $\pm$ 6.1%)
Method 2	---	---	10007 ± 301 (100.0 $\pm$ 3.0%)

Mean $\pm$ s.e.m. are shown.

Percent expected value are shown in ().

APPENDIX III

CALCULATION OF V_1 , K_{12} AND K_{21} (2-compartmental model)a. Calculation of V_1

After fitting a double exponential curve to data of the experiment KN-15, the following equation was obtained:

$$C^* = 13804.e^{-0.27383t} + 11688.e^{-0.013260t}$$

The number of dpm's injected was $M^*_0 = 32.264 \times 10^6$

$$\text{Hence } V_1 = \frac{32.263 \times 10^6}{13803 + 11688} = 1265.7 \text{ ml for a 12.4 kg dog.}$$

Thus for any dog weighing X kg, V_1 equals $\frac{(1265.7) \cdot (X)}{12.4}$

b. Calculation of K_{12} and K_{21}

Once solved, the differential form of the exponential equation mentioned in section a. gives for K_{12} :

$$K_{12} = \frac{\beta_1 B_2 + \beta_2 B_1}{B_1 + B_2} = \frac{(.27383 \cdot 11688) + (.01360 \cdot 13804)}{13804 + 11688}$$

$$K_{12} = 0.13273 \text{ min}^{-1}$$

According to Norwich (1977) and if $B_3 \neq B_1$:

$$K_{12} = \frac{B_1 B_2 (\beta_1 - \beta_2)}{B_3 (B_1 + B_2)}$$

$$\text{That is } B_3 = \frac{B_1 B_2 (\beta_1 - \beta_2)}{K_{12} (B_1 + B_2)} = \frac{(13804 \cdot 11688) (.27383 - .013260)}{.13273 (13804 + 11688)}$$

$$\text{and } B_3 = 12425.0$$

A third algebraic equation resulting from the substitution of the exponential equations for C^*_1 and C^*_2 into the differential equations for dC^*_1/dt and dC^*_2/dt is (Norwich, 1977):

$$K_{21} = \frac{B_3 (\beta_1 - \beta_2)}{B_1 + B_2} = \frac{12425.0 (.27383 - .01326)}{13804 + 11688}$$

$$\text{and } K_{21} = 0.12700$$

APPENDIX IV

STATISTICAL ANALYSIS OF RESULTS

TABLE 6
CONCENTRATION OF PLASMA GLUCAGON IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1656.75	1656.75	5.165 P < .05
between dogs	3	2065.21	688.40	2.146
interaction	3	791.14	263.71	.822
error	14	4490.50	320.75	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1628.03	1628.03	5.220 P < .05
between dogs	3	6115.63	2038.54	6.54 P < .025
interaction	3	488.43	162.81	.522
error	12	3740.33	311.69	

TABLE 7

CONCENTRATION OF PLASMA GLUCOSE IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.2668	.2668	128.51 P < .005
between dogs	3	.7117	.2372	114.25 P < .005
error	3	.0062	.0021	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.0010	.0010	.04
between dogs	3	.3610	.1200	5.10
error	3	.071	.0236	

TABLE 8

RATE OF APPEARANCE OF GLUCOSE (Ra) IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.5275	.5275	38.04 $P < .005$
between dogs	3	.0237	.0079	.57
error	3	.0416	.0139	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.1235	.1235	2.43
between dogs	3	.0652	.0217	.43
error	3	.1523	.0508	

TABLE 9

METABOLIC CLEARANCE RATE OF GLUCOSE (MCR) IN PHLORIZIN
INFUSED POSTABSORPTIVE DOGS.

TWO-WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.094	1.094	170.58 P < .005
between dogs	3	1.245	.415	64.67 P < .01
error	3	.019	.006	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.976	.976	89.44 P < .005
between dogs	3	.687	.229	20.98 P < .01
error	3	.033	.011	

TABLE 10

RATE OF APPEARANCE OF ALANINE (Ra-ALANINE) IN PHLORIZIN
INFUSED POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.081	.081	2.60
between dogs	3	2.234	.745	24.01 - P < .01
error	3	.093	.031	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.234	.234	14.20 P < .05
between dogs	3	2.786	.929	56.39 P < .01
error				

TABLE 11

RATE OF FORMATION OF GLUCOSE FROM ALANINE (GLUCOSE FROM ALANINE) IN PHLORIZIN INFUSED POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2.183	2.183	15.45 P < .01
between dogs	3	2.325	.775	5.48
error	3	.424	.141	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.011	.011	.70
between dogs	3	4.200	1.400	89.49 P < .005
error	3	.047	.016	

TABLE 12

FRACTION OF CIRCULATING GLUCOSE ARISING FROM ALANINE (% Ra
GLUCOSE FROM ALANINE) IN PHLORIZIN INFUSED POSTABSORPTIVE
DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.376	.376	12.36 P < .01
between dogs	3	1.317	.439	14.45 P < .05
error	3	.091	.030	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.002	.002	.02
between dogs	3	2.328	.776	10.91 P < .05
error	3	.213	.071	

TABLE 13.

FRACTION OF UTILIZED ALANINE-C TRANSFERRED TO GLUCOSE(% Rd ALANINE INTO GLUCOSE) IN PHLORIZIN INFUSED POSTABSORPTIVE DOGS.TWO WAY ANALYSIS OF VARIANCEA. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	7.303	7.303	78.84 P < .005
between dogs	3	1.693	.564	6.169
error	3	.274	.092	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.767	.767	13.86 P < .01
between dogs	3	2.603	.868	15.68 P < .01
error	3	.166	.055	

TABLE 14

CONCENTRATION OF PLASMA GLUCAGON IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	15600.19	15600.19	13.37 $P < .005$
between dogs	4	22320.30	5580.07	4.78 $P < .025$
interaction	4	5074.85	1268.71	1.09
error	18	21001.17	1166.73	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	46993.50	46993.50	24.12 $P < .005$
between dogs	4	132585.10	33146.28	17.02 $P < .005$
interaction	4	26140.30	6535.1	3.35
error	15	29218.17	1947.8	

TABLE 15

CONCENTRATION OF PLASMA GLUCOSE IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.499	1.499	49.00 $P < .005$
between dogs	4	.688	.172	5.69 $P < .05$
error	4	.121	.030	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.142	.142	12.99 $P < .005$
between dogs	4	.334	.086	7.90 $P < .05$
error	4	.044	.011	

TABLE 16

RATE OF APPEARANCE OF GLUCOSE (Ra) IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.153	.153	5.58 P < .05
between dogs	4	.054	.013	.48
error	4	.110	.027	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.090	.090	3.70
between dogs	4	.242	.060	2.48
error	4	.097	.024	

TABLE 17

METABOLIC CLEARANCE RATE OF GLUCOSE (MCR) IN PHLORIZIN
INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.547	.547	299.16 $P < .005$
between dogs	4	.630	.158	86.22 $P < .005$
error	4	.007	.002	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.312	1.312	73.44 $P < .005$
between dogs	4	.964	.241	13.49 $P < .01$
error	4	.071	.018	

Table 18

RATE OF APPEARANCE OF ALANINE (Ra-ALANINE) IN PHLORIZIN
INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.042	.042	.95
between dogs	4	2.621	.655	14.84
error	4	.177	.044	$P < .01$

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.099	.099	8.22
between dogs	4	2.130	.533	$P < .05$
error	4	.048	.012	44.16
				$P < .01$

TABLE 19

RATE OF FORMATION OF GLUCOSE FROM ALANINE (GLUCOSE FROM ALANINE) IN PHLORIZIN INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.049	3.049	20.66 $P < .01$
between dogs	4	5.865	1.466	9.94 $P < .05$
error	4	.590	.148	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.404	.404	5.57 $P < .05$
between dogs	4	11.867	2.964	40.85 $P < .01$
error	4	.290	.073	

TABLE 20

FRACTION OF CIRCULATING GLUCOSE ARISING FROM ALANINE (% Ra
GLUCOSE FROM ALANINE) IN PHLORIZIN INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2.057	2.057	10.05 $P < .05$
between dogs	4	8.738	2.184	10.67 $P < .05$
error	4	.819	.205	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.107	.107	1.74
between dogs	4	10.786	2.697	43.70 $P < .01$
error	4	.247	.062	

TABLE 21

FRACTION OF UTILIZED ALANINE-C GOING INTO GLUCOSE (% Rd
ALANINE INTO GLUCOSE) IN PHLORIZIN INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	12.273	12.273	53.30 $P < .005$
between dogs	4	1.059	.265	1.15
error	4	.921	.230	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2.827	2.827	18.53 $P < .01$
between dogs	4	3.600	.900	5.90 $P < .05$
error	4	.610	.152	

TABLE 22

CHANGE IN THE BASAL CONCENTRATION OF PLASMA GLUCAGON
BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($95 \leq t \leq 130$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	17.76	17.76	.10
between dogs	3	2241.57	747.19	4.28
interaction	3	593.78	197.93	$P < .05$ 1.13
error	13	2267.17	174.40	

TABLE 23

CHANGE IN THE BASAL CONCENTRATION OF PLASMA GLUCOSE
BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($95 \leq t \leq 130$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.049	.049	2.72
between dogs	3	.783	.261	14.43 $P < .05$
error	3	.054	.018	

TABLE 24

CHANGE IN THE BASAL RATE OF APPEARANCE OF GLUCOSE (Ra)
BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Phase 1 (95 ≤ t ≤ 130):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.393	.393	16.08 P < .01
between dogs	3	.040	.013	.54
error	3	.073	.024	

TABLE 25

CHANGE IN THE BASAL METABOLIC CLEARANCE RATE OF GLUCOSE
(MCR) BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED
STATE.

TWO WAY ANALYSIS OF VARIANCE

Phase 1 (95 ≤ t ≤ 130):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	1.269	1.269	13.94 P < .01
between dogs	3	.644	.215	2.36
error	3	.273	.091	

TABLE 26

CHANGE IN THE BASAL RATE OF FORMATION OF GLUCOSE FROM
ALANINE (GLUCOSE FROM ALANINE) BETWEEN DOGS IN THE
POSTABSORPTIVE AND IN THE FASTED STATE.
TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($95 \leq t \leq 130$):

Source of variation	degrees of freedom	sum of squares	mean square	F-value
between nutritional states	1	.043	.043	.56
between dogs	3	1.515	.505	6.64
error	3	.228	.076	$P < .05$

TABLE 27

CHANGE IN THE BASAL FRACTION OF CIRCULATING GLUCOSE ARISING
FROM ALANINE (% Ra GLUCOSE FROM ALANINE) BETWEEN DOGS IN
THE POSTABSORPTIVE AND IN THE FASTED STATE.
TWO WAY ANALYSIS OF VARIANCE

Phase 1 (95 ≤ t ≤ 130):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	2.461	2.461	17.53 P < .01
between dogs	3	1.904	.635	4.52
error	3	.421	.140	

TABLE 28

CHANGE IN THE BASAL FRACTION OF UTILIZED ALANINE-C GOING INTO
GLUCOSE (% Rd ALANINE INTO GLUCOSE) BETWEEN DOGS IN THE
POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Phase 1 ($95 \leq t \leq 130$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	3	.067	.067	.30
between dogs	3	.671	.224	.99
error	3	.676	.226	

TABLE 29

CHANGE IN THE INCREASED CONCENTRATION OF PLASMA GLUCAGON
FROM BASAL LEVEL DURING THE INFUSION OF PHLORIZIN BETWEEN
DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean / square	F value
between nutritional states	1	6000.00	6000.00	4.53 P < .05
between dogs	3	9615.00	3205.00	2.42
interaction	3	3178.00	1059.00	.8
error	16			

TABLE 30

CHANGE IN THE DECREASED CONCENTRATION OF PLASMA GLUCOSE
FROM BASAL LEVEL DURING THE INFUSION OF PHLORIZIN BETWEEN
DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.233	.233	11.46 P < .05
between dogs	3	.103	.034	1.68
error	3	.061	.020	

TABLE 31

CHANGE IN THE INCREASED RATE OF APPEARANCE OF GLUCOSE (Ra)
FROM BASAL LEVEL DURING THE INFUSION OF PHLORIZIN BETWEEN
DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.111	.111	2.53
between dogs	3	.004	.001	.028
error	3	.131	.044	

TABLE 32

CHANGE IN THE INCREASED METABOLIC CLEARANCE RATE OF GLUCOSE
(MCR) FROM BASAL LEVEL DURING THE INFUSION OF PHLORIZIN
BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.156	.156	9.90 $P < .05$
between dogs	3	.005	.002	104
error	3	.047	.016	

TABLE 33

CHANGE IN THE INCREASED RATE OF FORMATION OF GLUCOSE FROM
ALANINE (GLUCOSE FROM ALANINE) FROM BASAL LEVEL DURING THE
INFUSION OF PHLORIZIN BETWEEN DOGS IN THE P.A. AND FASTED STATE.

TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.018	.018	.10
between dogs	3	.978	.326	1.72
error	3	.570	.190	

TABLE 34

CHANGE IN THE INCREASED FRACTION OF CIRCULATING GLUCOSE ARISING
FROM ALANINE. (% Ra GLUCOSE FROM ALANINE) FROM BASAL LEVEL DURING
THE INFUSION OF PHLORIZIN BETWEEN DOGS IN THE P.A. AND FASTED STATE.
TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.645	.645	2.95
between dogs	3	.985	.328	1.501
error	3	.656	.219	

TABLE 35

CHANGE IN THE INCREASED FRACTION OF UTILIZED ALANINE-C GOING
INTO GLUCOSE (% Rd ALANINE INTO GLUCOSE) FROM BASAL LEVEL DURING
THE INFUSION OF PHLORIZIN BETWEEN DOGS IN THE P.A. AND FASTED STATE.
TWO WAY ANALYSIS OF VARIANCE

Source of variation	degrees of freedom	sum of squares	mean square	F value
between nutritional states	1	.00048	.00048	.02
between dogs	3	.657	.219	10.61 P < .05
error	3	.062	.021	

TABLE 36

CONCENTRATION OF PLASMA GLUCAGON IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$) :

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	5096	5096	9.01 $P < .01$
between dogs	4	63113	15778	27.90 $P < .005$
interaction	4	1124	281	.50
error	20	11309	565	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$) :

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	5352	5352	4.64 $P < .05$
between dogs	4	59240	14810	12.84 $P < .05$
interaction	4	1440	360	.31
error	15	17297	1153	

TABLE 37

CONCENTRATION OF PLASMA GLUCOSE IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.040	1.040	9.61 $P < .01$
between dogs	4	1.945	.486	4.49
error	4	.433	.108	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.152	.152	2.16
between dogs	4	3.430	.858	12.23 $P < .01$
error	4	.281	.070	

TABLE 38

RATE OF APPEARANCE OF GLUCOSE (Ra) IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	9.510	9.510	22.97 $P < .01$
between dogs	4	4.247	1.062	2.56
error	4	1.656	.414	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.560	1.560	43.56 $P < .005$
between dogs	4	11.100	2.780	77.27 $P < .005$
error	4	.144	.036	

TABLE 39

METABOLIC CLEARANCE RATE OF GLUCOSE (MCR) IN PHLORIZIN
INFUSED POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.224	3.224	14.60 P < .01
between dogs	4	1.935	.484	2.19
error	4	.883	.221	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.200	3.200	14.14 P < .01
between dogs	4	10.078	2.519	11.13 P < .01
error	4	.905	.226	

TABLE 40

RATE OF GLYCOGENOLYSIS IN PHLORIZIN INFUSED POSTABSORPTIVE
DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	5.497	5.497	16.87 P < .01
between dogs	4	1.160	.290	.89
error	4	1.303	.326	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.696	.696	.92
between dogs	4	6.427	1.607	2.11
error	4	3.041	.760	

TABLE 41

RATE OF RECIRCULATION OF ^{14}C -ATOM INTO GLUCOSE (GLUCOSE BY
RECIRCULATION) IN PHLORIZIN INFUSED POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.162	3.162	26.32 $P < .01$
between dogs	4	.414	.103	.86
error	4	.481	.120	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.140	1.140	4.97
between dogs	4	4.749	1.187	5.17
error	4	.918	.230	

TABLE 42

CONCENTRATION OF PLASMA GLUCOSE IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.264	.264	104.98 P < .005
between dogs	3	.064	.021	8.43 P < .05
error	3	.008	.002	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.002	.002	.34
between dogs	3	.063	.021	2.95
error	3	.021	.007	

TABLE 43

RATE OF APPEARANCE OF GLUCOSE (Ra) IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2.439	2.439	40.44 $P < .005$
between dogs	3	2.842	.947	15.71 $P < .05$
error	3	.181	.060	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.640	.640	3.54
between dogs	3	3.840	1.280	7.09 $P < .05$
error	3	.542	.181	

TABLE 44

METABOLIC CLEARANCE RATE OF GLUCOSE (MCR) IN PHLORIZIN
INFUSED POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.257	1.257	32.75 P < .005
between dogs	3	1.306	.435	11.34 P < .05
error	3	.115	.038	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2.491	2.491	33.04 P < .005
between dogs	3	3.534	1.178	15.63 P < .05
error	3	.226	.075	

TABLE 45

CONCENTRATION OF PLASMA GLYCEROL IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.384	1.384	2.78
between dogs	3	1.984	.661	1.330
error	3	1.491	.497	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.778	.778	2.94
between dogs	3	4.212	1.404	5.31
error	3	.793	.264	

TABLE 46

RATE OF APPEARANCE OF GLYCEROL (Ra-GLYCEROL) IN PHLORIZIN
INFUSED POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.712	.712	11.21 P < .05
between dogs	3	.288	.096	1.51
error	3	.190	.064	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.005	.005	.10
between dogs	3	.325	.108	1.97
error	3	.165	.055	

TABLE 47

RATE OF FORMATION OF GLUCOSE FROM GLYCEROL (GLUCOSE FROM GLYCEROL) IN PHLORIZIN INFUSED POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.183	3.183	35.92 P < .005
between dogs	3	.307	.102	1.16
error	3	.266	.089	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.212	.212	.73
between dogs	3	.631	.210	.72
error	3	.876	.292	

TABLE 48

FRACTION OF CIRCULATING GLUCOSE ARISING FROM GLYCEROL($\frac{1}{2}$ Ra-GLUCOSE FROM GLYCEROL) IN PHLORIZIN INFUSEDPOSTABSORPTIVE DOGS.TWO WAY ANALYSIS OF VARIANCEA. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2.621	2.621	24.40 $P < .01$
between dogs	3	.691	.230	2.14
error	3	.322	.107	

B. Phase 2 ($130 \leq t \leq 200$), vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.070	.070	.13
between dogs	3	1.539	.513	.97
error	3	1.591	.530	

TABLE 49

FRACTION OF UTILIZED GLYCEROL-C TRANSFERRED TO GLUCOSE
(% Rd-GLYCEROL INTO GLUCOSE) IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.
TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.024	.024	.10
between dogs	3	1.628	.542	2.17
error	3	.750	.250	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.021	.021	.15
between dogs	3	1.516	.505	3.57
error	3	.424	.142	

TABLE 50

CONCENTRATION OF PLASMA LACTATE IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.184	.184	1.67
between dogs	3	6.310	2.103	19.04 P < .01
error	3	.331	.110	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.408	1.408	6.61 P < .05
between dogs	3	6.626	2.208	10.36 P < .05
error	3	.639	.213	

TABLE 51

SPECIFIC ACTIVITY OF LACTATE (SA-LACTATE) IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.023	.023	.15
between dogs	3	4.293	1.431	9.29 $P < .05$
error	3	.462	.154	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.100	.100	2.35
between dogs	3	3.462	1.154	27.01 $P < .01$
error	3	.128	.043	

TABLE 52

CONCENTRATION OF PLASMA GLUCAGON IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	6348	6348	21.08 P < .005
between dogs	3	17125	5708	18.95 P < .005
interaction	3	2010	670	2.25
error	14	4216	301	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2800	2800	5.28 P < .05
between dogs	3	2776	925	1.74
interaction	3	1879	626	1.18
error	11	5835	530	

TABLE 53

CONCENTRATION OF PLASMA ACTH IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	5.227	5.227	1.07
between dogs	2	208.232	104.116	21.39 P < .005
interaction	2	16.961	8.480	1.74
error	12	58.403	4.866	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	62.333	62.333	4.84
between dogs	2	119.137	59.568	4.63 P < .05
interaction	2	59.288	29.644	2.30
error	9	115.885	12.876	

TABLE 54 .

CONCENTRATION OF PLASMA CORTISOL IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.197	3.197	1.28
between dogs	3	89.379	29.793	11.91 $P < .01$
interaction	3	4.501	1.500	.60
error	16	40.029	2.501	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	26.573	26.573	6.73 $P < .05$
between dogs	3	160.557	53.519	13.54 $P < .01$
interaction	3	18.205	6.068	1.54
error	12	47.403	3.950	

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CONCENTRATION OF PLASMA EPINEPHRINE IN PHLORIZIN INFUSED
POSTABSORPTIVE DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	801	801	.22
between dogs	3	96764	32255	8.94
interaction	3	10540	3513	P < .01 .97
error	12	43310	3609	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1463	1463	.23
between dogs	3	135471	45157	7.07
interaction	3	19052	6351	P < .01 .99
error	12	76620	6385	

TABLE 56

CONCENTRATION OF PLASMA GLUCOSE IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.114	1.114	37.57 $P < .005$
between dogs	3	.399	.133	4.490
error	3	.089	.030	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.040	.040	1.03
between dogs	3	1.377	.459	11.63 $P < .05$
error	3	.118	.039	

TABLE 57

RATE OF APPEARANCE OF GLUCOSE (Ra) IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.641	3.641	26.48 P < .01
between dogs	3	.053	.018	.13
error	3	.412	.138	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.773	.773	68.69 P < .005
between dogs	3	.846	.282	25.06 P .01
error	3	.034	.011	

TABLE 58

METABOLIC CLEARANCE RATE OF GLUCOSE (MCR) IN PHLORIZIN
INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.303	1.303	20.84 $P < .01$
between dogs	3	.403	.134	2.15
error	3	.188	.062	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	2.263	2.263	49.06 $P < .005$
between dogs	3	2.015	.672	14.56 $P < .05$
error	3	.138	.046	

TABLE 59

CONCENTRATION OF PLASMA GLYCEROL IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	4.057	4.057	3.45
between dogs	3	3.660	1.220	1.037
error	3	3.529	1.176	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	3.569	3.569	2.44
between dogs	3	24.406	8.135	5.57
error	3	4.381	1.460	

TABLE 60

RATE OF APPEARANCE OF GLYCEROL (Ra-GLYCEROL) IN PHLORIZIN
INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.963	.963	4.75
between dogs	3	4.076	1.359	6.71 $P < .05$
error	3	.608	.202	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.306	1.306	1.39
between dogs	3	9.558	3.186	3.40
error	3	2.814	.938	

TABLE 61

RATE OF FORMATION OF GLUCOSE FROM GLYCEROL (GLUCOSE FROM GLYCEROL) IN PHLORIZIN INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	8.570	8.570	7.338 P < .05
between dogs	3	5.081	1.694	1.450
error	3	3.503	1.168	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	9.266	9.266	1.062
between dogs	3	68.055	22.685	2.601
error	3	26.167	8.722	

TABLE 62

FRACTION OF CIRCULATING GLUCOSE ARISING FROM GLYCEROL
($\text{\%}$ Ra-GLUCOSE FROM GLYCEROL) IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	6.106	6.106	3.44
between dogs	3	14.147	4.716	2.66
error	3	5.326	1.776	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.680	.680	.51
between dogs	3	39.342	13.114	9.89 P .05
error	3	3.976	1.325	

TABLE 63

FRACTION OF UTILIZED GLYCEROL-C TRANSFERRED TO GLUCOSE
(% Rd-GLYCEROL INTO GLUCOSE) IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	1.315	1.315	6.64 P < .05
between dogs	3	3.241	1.080	5.45
error	3	.594	.198	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.013	.013	2.84
between dogs	3	6.645	2.215	488.84 P < .005
error	3	.014	.004	

TABLE 64

CONCENTRATION OF PLASMA LACTATE IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.240	.240	3.21
between dogs	3	4.627	1.542	20.70
error	3	.224	.074	$P < .01$

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.297	.297	.71
between dogs	3	1.469	.490	1.16
error	3	1.261	.420	

TABLE 65

SPECIFIC ACTIVITY OF LACTATE (SA-LACTATE) IN PHLORIZIN
INFUSED FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.334	.334	7.48 $P < .05$
between dogs	3	.319	.106	2.38
error	3	.134	.045	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	.248	.248	.75
between dogs	3	1.283	.428	1.30
error	3	.991	.330	

TABLE 66

CONCENTRATION OF PLASMA GLUCAGON IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	11456	11456	11.44 $P < .01$
between dogs	3	16536	5512	5.51 $P < .05$
interaction	3	2049	683	.68
error	15	15016	1001	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	24424	24424	19.99 $P < .01$
between dogs	3	24401	7467	5.96 $P < .05$
interaction	3	2460	820	.65
error	12	15035	1253	

TABLE 67

CONCENTRATION OF PLASMA ACTH IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	253.500	253.500	20.41 $P < .005$
between dogs	2	252.756	126.378	10.17 $P < .01$
interaction	2	89.852	44.926	3.62
error	12	149.055	12.421	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	39.151	39.151	1.21
between dogs	2	31.549	15.774	.49
interaction	2	82.481	41.240	1.28
error	9	291.098	32.344	

TABLE 68

CONCENTRATION OF PLASMA CORTISOL IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	61.856	61.856	4.73 $P < .05$
between dogs	3	191.075	63.691	4.87 $P < .05$
interaction	3	36.635	12.211	.93
error	16	209.276	13.079	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	26.096	26.096	1.86
between dogs	3	339.622	113.207	8.06 $P < .01$
interaction	3	44.018	14.672	1.04
error	12	168.47	14.039	

TABLE 69

CONCENTRATION OF PLASMA EPINEPHRINE IN PHLORIZIN INFUSED
FASTED DOGS.

TWO WAY ANALYSIS OF VARIANCE

A. Phase 1 ($95 \leq t \leq 130$) vs Phase 2 ($130 \leq t \leq 200$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	12843	12843	2.33
between dogs	3	453865	151288	27.46 P < .005
interaction	3	36498	12166	2.21
error	12	71614	5509	

B. Phase 2 ($130 \leq t \leq 200$) vs Phase 3 ($200 \leq t \leq 240$):

Source of variation	degrees of freedom	sum of squares	mean square	F value
between phases	1	28428	28428	1.21
between dogs	3	446483	148828	6.33 P < .01
interaction	3	37480	12493	.53
error	12	282264	23522	

TABLE 70

CHANGE IN THE BASAL LEVEL OF PLASMA GLUCOSE (C), RATE OF APPEARANCE (Ra) AND METABOLIC CLEARANCE RATE (MCR) OF GLUCOSE, PLASMA GLYCEROL (G) AND RATE OF APPEARANCE OF GLYCEROL (Ra-G), BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Expt.no.	ΔC	ΔRa	ΔMCR	ΔG	$\Delta Ra-G$
1	0.2601	0.9260	0.0383	0.8963	0.3784
2	-0.0120	1.0523	0.4399	1.5417	0.4328
3	-0.1777	0.2367	0.1443	0.2474	1.3696
4	-0.0163	1.9972	0.8863	1.0752	0.2500
Mean	0.0135	1.0531	0.3772	0.9402	0.6077
s.e.m.	0.0908	0.3622	0.1898	0.2680	0.2568
t	0.8484	2.9076	1.7206	3.5076	2.3660
p <		.05 _{1-tail}	.10 _{1-tail}	.05	.05

TABLE 71

CHANGE IN THE BASAL RATE OF FORMATION OF GLUCOSE FROM GLYCEROL (FG), IN THE BASAL FRACTION OF CIRCULATING GLUCOSE ARISING FROM GLYCEROL (CG), IN THE BASAL FRACTION OF UTILIZED GLYCEROL-C GOING INTO GLUCOSE (GC), IN THE BASAL PLASMA LACTATE (L) AND IN THE BASAL SPECIFIC ACTIVITY OF LACTATE (SA-L), BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Expt.no.	Δ FG	Δ CG	Δ GC	Δ L	Δ SA-L
1	0.3043	0.8770	2.5370	0.2847	0.8696
2	0.6745	0.7926	0.9683	-0.5180	0.5988
3	0.1948	-0.4164	-0.0740	0.6547	-0.1851
4	0.0411	-0.4926	0.7855	-0.6426	-0.2791
Mean	0.3037	0.1902	1.0542	-0.0553	0.2510
s.e.m.	0.1349	0.3729	0.5440	0.3134	0.2850
t	2.2514	0.1066	1.9379	0.1764	0.8808

P <

TABLE 72

CHANGE IN THE BASAL CONCENTRATION OF PLASMA GLUCAGON, ACTH,
CORTISOL AND EPINEPHRINE BETWEEN DOGS IN THE POSTABSORPTIVE
AND IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Expt.no.	Δ GLUCAGON	Δ ACTH	Δ CORTISOL	Δ EPINEPHRINE
1	59.2	2.50	1.27	266.6
2	11.8	-	3.50	- 25.0
3	- 3.0	-10.60	3.26	-132.0
4	-14.7	7.42	1.37	0.0
Mean	13.3	- 0.23	2.35	27.4
s.e.m.	16.2	5.38	0.60	84.7
t	0.82	0.04	3.94	0.32
p <			.05	

TABLE 73

CHANGE IN THE DECREASED CONCENTRATION OF PLASMA GLUCOSE (C),
IN THE INCREASED RATE OF APPEARANCE (Ra) AND METABOLIC
CLEARANCE RATE (MCR) OF GLUCOSE, IN THE INCREASED PLASMA
GLYCEROL (G) AND RATE OF APPEARANCE OF GLYCEROL (Ra-G)
FROM BASAL LEVEL DURING THE INFUSION OF PHLORIZIN BETWEEN
DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Expt.no.	ΔC	ΔRa	ΔMCR	ΔG	$\Delta Ra-G$
1	0.5814	0.0746	0.3151	1.4289	0.3802
2	0.4676	0.0720	0.0402	0.6412	-0.5750
3	0.2910	0.1284	0.0431	0.9592	0.9958
4	0.1902	0.7048	-0.3404	-0.6599	-0.4019
Mean	0.3826	0.2450	0.0145	0.5924	0.0948
s.e.m.	0.0876	0.1538	0.1347	0.4477	0.3626
t	4.3653	1.5953	0.1076	1.3232	0.2614
p <	.05				

TABLE 74

CHANGE IN THE INCREASED RATE OF FORMATION OF GLUCOSE FROM GLYCEROL (FG), IN THE FRACTION OF CIRCULATING GLUCOSE ARISING FROM GLYCEROL (CG) AND IN THE FRACTION OF UTILIZED GLYCEROL-C GOING INTO GLUCOSE (GC) FROM BASAL LEVEL DURING THE INFUSION OF PHLORIZIN BETWEEN DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

PAIRED STUDENT 'S t TEST

Expt.no.	Δ FG	Δ CG	Δ GC
1	2.1133	1.3760	1.5298
2	-1.1922	-1.4561	-0.0563
3	2.5448	3.5704	0.4569
4	-0.2320	-0.0797	0.8727
Mean	0.8085	0.8526	0.7008
s.e.m.	0.9038	1.0746	0.3353
t	0.8943	0.5143	2.0897

p <

TABLE 75

CHANGE IN THE INCREASED CONCENTRATION OF PLASMA GLUCAGON
FROM BASAL LEVEL DURING THE INFUSION OF PHLORIZIN BETWEEN
DOGS IN THE POSTABSORPTIVE AND IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Expt.no.	Δ GLUCAGON
1	15.2
2	38.2
3	45.0
4	31.7
Mean	32.5
s.e.m.	6.4
t	5.1
p <	.02

APPENDIX V

QUALITY CONTROL

TABLE 76

CONCENTRATION OF PLASMA GLUCOSE DUPLICATES IN PHLORIZIN
INFUSED DOG IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Sample no.	Δ concentration
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1	1
2	-2
3	-2
4	2
5	2
6	-2

Mean	-0.17
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s.e.m.	0.83
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t	0.20
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p	
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TALBE 77

OPTICAL DENSITY OF PLASMA ALANINE DUPLICATES IN PHLORIZIN
INFUSED DOG IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Sample no. Δ O.D.

1 .010

2 -.005

3 .000

4 -.024

5 -.010

6 .006

7 -.007

Mean -.004

s.e.m .004

t 1.349

p

TABLE 78

OPTICAL DENSITY (O.D.) OF PLASMA GLYCEROL DUPLICATES
IN PHLORIZIN INFUSED DOG IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Sample no.	Δ O.D.
1	.004
2	.006
3	-.002
4	-.004
5	-.001
6	.002
7	-.003
Mean	.0003
s.e.m.	.0014
t	.200
p	

TABLE 79

OPTICAL DENSITY (O.D.) OF PLASMA LACTATE DUPLICATES
IN PHLORIZIN INFUSED DOG IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Sample no. Δ O.D.

1 -.005

2 -.002

3 -.016

4 .002

5 -.010

6 -.002

Mean -.005

s.e.m. .002

t .046

p

TABLE 80

COUNTS (CPM) OF PLASMA GLUCAGON DUPLICATES IN PHLORIZIN
INFUSED DOG IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Sample no.	Δ CPM
8	2
10	-1
11	-8
13	-2
15	-1
16	2
Mean	-1.33
s.e.m.	1.50
t	.89
p	

TABLE 81

CONCENTRATION OF PLASMA EPINEPHRINE DUPLICATES IN PHLORIZIN
INFUSED DOG IN THE FASTED STATE.

PAIRED STUDENT'S t TEST

Sample no. Δ concentration

8 7

10 2

11 -7

13 -4

15 0

16 -2

Mean -0.66

s.e.m. 1.99

t 0.334

p

TABLE 82

CONCENTRATION OF PLASMA ACTH DUPLICATES IN PHLORIZIN
INFUSED DOG IN THE POSTABSORPTIVE STATE.

PAIRED STUDENT'S t TEST

Sample no. Δ concentration

8 -1.564

10 -0.196

11 -0.460

13 0.051

15 0.057

16 -0.264

18 -0.414

Mean -0.399

s.e.m. .209

t 1.909

p

TABLE 83

CONCENTRATION OF PLASMA CORTISOL DUPLICATES IN PHLORIZIN
INFUSED DOG IN THE POSTABSORPTIVE STATE.

PAIRED STUDENT'S t TEST

Sample no.	Δ concentration
6	-0.44
8	0.14
10	3.08
11	0.29
13	0.29
15	0.58
17	0.30
18	-0.06
Mean	0.522
s.e.m.	0.381
t	1.373
p	