EFFECTS OF INTRAVENOUS AND ORAL INFUSION OF MONOSACCHARIDES ON SERUM INSULIN LEVELS IN RABBITS

A THESIS PRESENTED TO THE

FACULTY OF GRADUATE STUDIES AND RESEARCH

OF THE

UNIVERSITY OF MANITOBA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

BY

MOHINDER SINGH NIJJAR

1968

DEDICATED TO

MY PARENTS, SISTERS AND BROTHER

ACKNOWLEDGMENT

The author expresses gratitude to Dr. W. F. Perry for the guidance and encouragement which he provided throughout this study. I also thank Drs. M. C. Blanchaer and R. J. Hoeschen for their invaluable criticism.

TABLE OF CONTENTS

ABSTRACT 1

Organization of Thesis 3

SECTION I

A. GENERAL INTRODUCTION 4

B. PURPOSE 5

C. GENERAL APPROACH 6

SECTION II

REVIEW OF LITERATURE

A. DISCOVERY, BIOSYNTHESIS, STORAGE AND SECRETION OF INSULIN 7

B. STIMULI FOR INSULIN SECRETION 7

1) Monosaccharides and related substances 7

a) Sugar transport 9

b) Phosphorylation of sugar 9

c) Glycolysis 10

d) Hexose monophosphate shunt 10

e) Metabolites 10

f) Amino acids 11

2) Intestinal Hormones 11

a) Secretin 12

b) Pancreozymin 14

3) Other Hormones 15

a) Glucagon 15

b) Gastrin 17

4) Nervous Stimulu 17

a) Stimulation of vagus nerve 17

C. INHIBITORS OF INSULIN SECRETION 17

1) Epinephrine 17

2) Norepinephrine 18

SECTION III

MATERIALS EXPERIMENTAL PROCEDURES AND METHODOLOGY

A. MATERIALS 19

1) Animals 19

2) Anesthesia 19

3) Test substances and dosage used 19

4) Control experiments 19

B. EXPERIMENTAL PROCEDURES 19

1) Design of experiments 19

2) Fasting and Anesthesia 20

3) Intravenous tests 20

4) Oral tests 20

5) Control tests 20

C. METHODS OF ANALYSIS 21

1) Blood sugars analysis 21

2) Assay of Insulin 21

D. PARAMETERS 21

E. PRESENTATION OF RESULTS 22

F. STATISTICAL ANALYSIS 22

SECTION IV

RESULTS

A. EFFECTS OF INTRAVENOUSLY ADMINISTERED MONOSACCHARIDES AND OTHER SUBSTANCES ON SERUM INSULIN AND BLOOD GLUCOSE LEVELS

1) Saline 24

2) Glucose 24

3) Mannose 24

4) Fructose 28

5) Ribose 28

6) Galactose 31

7) Xylose 31

8) ∝-methyl Glucoside 31

9) Pyruvate 35

B. COMPARISON OF INSULIN RESPONSES TO INTRAVENOUS MONOSACCHARIDES

1) Mannose and Fructose 35

2) Glucose 38

3) Galactose 38

4) Xylose and Ribose 38

5) Pyruvate 39

6) ∝-methyl Glucoside 39

C. EFFECTS OF ORALLY INFUSED MONOSACCHARIDES AND OTHER SUBSTANCES ON SERUM INSULIN AND BLOOD GLUCOSE LEVELS 39

1) Preliminary experiments 40

2) Saline 42

3) Glucose 42

4) Mannose 42

5) Fructose 46

6) Ribose 46

7) Galactose 49

8) Xylose 49

9) ∝-methyl Glucoside 52

10) Glycine 52

SECTION V

DISCUSSION

A. EXPERIMENTAL PROCEDURES 56

1) Controls 56

2) Rapid Infusion of sugars 56

B. INTRAVENOUS ADMINISTRATION OF MONOSACCHARIDES 56

1) Classification of Monosaccharides 57

2) Specificity of Insulin Secretion 59

3) Discussion of Insulin Responses Individually 60

i) Glucose 60

ii) Mannose 60

iii) Fructose 61

iv) Galactose 61

v) Ribose 62

vi) Xylose 64

vii) ∝-methyl Glucoside 64

viii) Pyruvate 64

4) Conclusions 65

C. ORAL ADMINISTRATION OF MONOSACCHARIDES 65

1) Discussion of Insulin Responses Individually 65

i) Glucose 65

ii) Fructose 66

iii) Mannose 67

iv) Galactose 67

v) Xylose 68

vi) Ribose 69

vii) ∝-methyl Glucoside 69

viii) Glycine 69

2) Comparison of Oral and Intravenous Insulin Responses 71

3) Specificity 72

4) Conclusions 73

SECTION VI

BIBLIOGRAPHY 75-83

SECTION VII

APPENDIX A - METHODOLOGY 86

1) Hydrolysis of ∝-methyl Glucoside 86

2) Preparation of Reagents 86

i) Borate Buffer 86

ii) Bovine Serum Albumin Solution (B.S.A.) 86

iii) Standards of Pork Insulin 87

iv) Anti insulin antibody-guinea pig (AIS-GP) Solution 87

v) Insulin-I^{125} Solution 88

vi) Purification of Resin 88

3) Immunoassay of Insulin 88

i) Principle 88

ii) Serum Separation 88

iii) Procedure 88

iv) Recovery 89

v) Immunoassay in Summary 90

APPENDIX B - STATISTICAL ANALYSIS 92

1) Analysis of Variance (single classification)[1] 92

APPENDIX C - TABLES CONTAINING EXPERIMENTAL DATA 94-142

LIST OF TABLES

I	Effects of Rapid Intavenous Infusion of Saline on Serum Insulin and Blood Glucose Concentration in Rabbits	95
II	Effects of Intavenous Infusion of D(+) Glucose (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Sugar Levels in Rabbits.	97
III	Effects of Intavenous Infusion of D(+) Mannose (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Levels in Rabbits.	99
IV	Effects of Intravenous Administration of D-Fructose (1.5 gm./ 3cc's of solution/kg. b. wt.) on Serum Insulin Levels and Blood Glucose Concentration in Rabbits.	101
V	Effects of Rapid Intravenous Injection of D(-) Ribose (1.5. gm./ 3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Levels in Rabbits.	103
VI	Effect of Rapid Intravenous Infusion of D(+) Galactose (1.5 gm./ 3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits.	105
VII	Effects of Intravenous Infusion of D-Xylose (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Levels in Rabbits.	107
VIII	Effect of Rapid Intravenous Infusion of α-Methyl Glucoside (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Levels in Rabbits.	109

IX	Effect of Intravenous Infusion of Pyruvate (Na+) over 5 minutes (0.91 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits.	111
X	Comparison of Decay Constant (K) of Various Monosaccharides Infused Intravenously*	113
XI	Comparison of maximum increases in Serum Insulin Levels after the Intravenous Infusion of Different Monosaccharides*	115
XII	Effects of Rapid Intravenous Administration of D(+) Glucose (400 mgs./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits.	117
XIII	Effects of Rapid Administration of Physiological Saline (3cc's of saline/kg.b.wt.) Intragastrically (Orally) on Serum Insulin and Blood Glucose Concentrations in Rabbits.	119
XIV	Effects of Rapid Oral Administration of D(+) Glucose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits.	121
XV	Effects of Oral Administration of D(+) Mannose Rapidly (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits.	123
XVI	Effects of Oral Infusion of D-Fructose (1.5 gm./3cc's of solution/kg.b.wt.) Rapidly on Serum Insulin and Blood Glucose Concentrations in Rabbits.	125
XVII	Effect of Rapid Oral Administration of D(-) Ribose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits.	127

XVIII Effect of Rapid Oral Administration of D(+) Galactose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentration in Rabbits. 129

XIX Effect of Rapid Oral Administration of D-Xylose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentration in Rabbits. 131

XX Effects of Oral Infusion of ∝-methyl Glucoside Rapidly (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits. 133

XXI Effects of Rapid Oral Administration of Glycine (Equimoles 0.62 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Sugar Levels in Rabbits. 135

XXII Comparison of Maximum Increases in Serum Insulin Levels (Intravenously verus Oral) and in Actual Blood Sugar Concentration (Intravenous versus Oral). 137

XXIII Comparison of Present Findings With the Previously Reported Data 139

LIST OF FIGURES

1. Effects of rapid intravenous infusion of saline on blood sugar and serum insulin levels. 25

2. Effects of rapid intravenous infusion of D(+) Glucose on blood glucose and serum insulin levels. 26

3. Effects of rapid intravenous infusion of D(+) Mannose on true blood glucose, blood mannose and serum insulin levels. 27

Inplot. Shows a semilogarithmic plot of blood mannose concentration versus time in minutes after the infusion.

4. Effects of rapid intravenous infusion of D-Fructose on true blood glucose, blood fructose and serum insulin levels. Inplot. 29

Shows a semilogarithmic plot of blood fructose levels versus time in minutes after the infusion.

5. Effects of rapid intravenous infusion of D(-) Ribose on true blood glucose, blood ribose and serum insulin levels. 30

Inplot. Shows a semilogarithmic plot of blood ribose concentration at different time intervals after the infusion.

6. Effects of rapid intravenous infusion of D(+) Galactose on true blood glucose, blood galactose and serum insulin levels. 32

Inplot. Shows the rate of removal of intravenous galactose.

7. Effects of rapid intravenous infusion of D-Xylose on true blood glucose, blood xylose and serum insulin levels. 33

Inplot. Shows a semilogarithmic plot of blood xylose levels versus time in minutes after the infusion.

8. Effects of rapid intravenous infusion of ∝-methyl Glucoside on true blood glucose, blood ∝-methyl glucoside and serum insulin levels. 34

Inplot. Shows the rate of removal of intravenous ∝-methyl glucoside.

9. Effects of slow intravenous infusion of Pyruvate (Equimoles to monosaccharides) on true blood glucose, and serum insulin levels. 36

10. Histogram depicting the decay constant (K) of different monosaccharides. 37

11. Histogram showing the maximum rises in serum insulin levels following the infusion of various monosaccharides. 37

12. Histogram showing the effects of Glucose infused intraduodenally and orally on the blood sugar and serum insulin levels in Rabbits. 41

13. Effects of rapid oral administration of saline on blood sugars and serum insulin levels. 43

14. Effects of rapid oral administration of D(+) Glucose on blood glucose and serum insulin levels. 44

15. Effects of rapid oral administration of D(+) Mannose on true blood glucose, blood mannose and serum insulin levels. 45

16. Effects of rapid oral administration of D-Fructose on true blood glucose, blood fructose and serum insulin levels. 47

17. Effects of rapid oral administration of D(-) Ribose on true blood glucose, blood ribose and serum insulin levels. 48

18. Effects of rapid oral administration of D(+) Galactose on true blood glucose, blood galactose and serum insulin levels. 50

19. Effects of rapid oral administration of D-Xylose on true blood glucose, blood xylose and serum insulin levels. 51

20. Effects of rapid oral administration of ∝-methyl Glucoside on true blood bglucose, blood ∝-methyl glucoside and serum insulin levels. 53

21. Effects of oral administration of Glycine (Equimoles to monosaccharides) on blood sugars and serum insulin levels. 54

22. Depicts the comparison of maximum increases in serum insulin levels (intravenous versus oral) and in actual blood sugar concentration (intravenous versus oral). 70

ABSTRACT

EFFECTS OF INTRAVENOUS AND ORAL INFUSION OF MONOSACCHARIDES ON SERUM INSULIN LEVELS IN RABBITS by M.S. Nijjar.

Glucose, Fructose, Mannose, Galactose, α-methyl Glucoside, Ribose and Xylose were administered intravenously and orally and their effects on serum levels of immunoreactive insulin were observed. Pyruvate (intravenous) and Glycine (oral) were also investigated. Intravenously, all the sugars except α-methyl glucoside stimulated insulin secretion to varying degrees. With the exception of glucose, the insulin response to all sugars was not associated with any change in true blood glucose concentration. Pyruvate had a slight stimulatory effect on insulin secretion. The phenomenon of insulin secretion seems to be associated with an active reducing group of a sugar molecule but was not related to the molecular arrangement at carbon #2 to #6. The insulin response was related to the rate of metabolism of the sugar infused; most readily metabolizable sugars - glucose, fructose and mannose produced immediate and marked insulin responses whereas a delayed and moderate insulin response followed poorly metabolizable sugars - galactose, xylose and ribose. No insulin response occurred after a non-metabolizable sugar - α-methyl glucoside.

Oral infusion of glucose, galactose, mannose, fructose, xylose and ribose enhanced the release of insulin. α-methyl glucoside and glycine had no stimulatory effect on insulin secretion. The insulin response to these sugars was similar to that after intravenous infusion despite a very much lower blood level of the various sugars following their oral administration.

The previously reported mechanism i.e. the release of an insulinotropic

substance in response to oral glucose, is not specific for glucose. Furthermore, it does not seem to respond to 1) the presence of a nutrient in the gut and 2) the rate and the mechanism of glucose transport in the small intestine.

Organization of Thesis

This thesis is divided into sections. Section 1 contains the general introduction, purpose and the general approach of this study. Section 11 includes a review of the literature dealing briefly with the discovery, biosynthesis and secretion of insulin and in more detail with the stimulation of insulin secretion. The inhibitors of insulin secretion are also briefly discussed. Section 111 is concerned with the materials, experimental procedures and in brief, the analytical methods. The parameters used, the manner of presenting the results and statistical procedures are also described. The findings of intravenous and oral administration of monosaccharides are presented in Section IV. Section V includes discussion of the results and conclusions. Section VI contains the bibliography. Section VII contains the appendices giving details of the analytical methods and tables containing the data obtained from this study.

SECTION I

A. GENERAL INTRODUCTION

The blood glucose level represents a balance between the rate at which glucose is entering the blood stream from the liver and exogenous sources, and the rate at which it is being removed from the blood stream by the peripheral tissues. Insulin plays an important role in maintaining such a homeostasis (1). This hormone is secreted by β-cells of the pancreas in response to a number of stimuli and lowers the blood glucose concentration by enhancing its utilization in the peripheral tissues and uptake by the liver. Other hormones such as epinephrine, glucagon, growth hormone and glucocorticoids raise the blood glucose concentration either by inhibiting its peripheral utilization, or by enhancing glycogenolysis or by gluconeogenesis (3).

The phenomenon of insulin secretion by the pancreas and its action in the peripheral tissues has been reviewed by Levine (2) and by Williams (3). It is known that a rise in the blood glucose concentration stimulates insulin secretion. However, it has been shown that hyperglycemias produced by the intravenous infusions of different sugars, differ considerably in their insulinotropic effect (6 -12). Other alleged stimuli to insulin secretion are glucagon (42, 44 - 47), secretin (35 -38), pancreozymin (37, 40), various metabolites such as TCA cycle intermediates, amino acids, ketone bodies (15 - 21), certain cations such as K^+, Ca^{++} or Mg^{++} (54, 55) and stimulation of the vagus nerve (52, 53). The mechanism by which these stimuli bring about insulin secretion is not fully understood as yet.

In the past, very little attention has been paid to the specificity of insulin secretory mechanism with the exception of brief mention in two reports. In 1958,

Pozza et al. (6) investigated this phenonmenon in dogs by means of cross-circulation experiments. However, they utilized the extent of hypoglycemia produced in the recipient dog as an index of insulin secretion. In 1963, Grodsky et al. (9) studied this process by perfusing the isolated rat pancreas. However, it remained of considerable interest to investigate the specificity of this phenomenon in vivo and by measuring the immunoreactive insulin.

Various investigators have shown that the administration of glucose either orally (34) or intraduodenally (32, 33) stimulated a greater release of insulin than if the same amount of glucose was injected intravenously. Based upon these findings, it was suggested that an insulinotropic substance was released from the small intestine during the absorption of glucose which stimulated the release of insulin in addition to the stimulation of the pancreas by an increase in the blood glucose concentration. It remained of an interest, however, to determine if such differences in the insulin response are specific to glucose or if they can be brought about by the infusion of other sugars as well. Goetz et al. (11) have also suggested the possibility of a mechanism in the hepato-portal system which stimulated insulin secretion in response to changes in the portal blood glucose concentration.

B. PURPOSE

The object of the present study was threefold:

1) to determine the specificity of insulin secretory mechanism to the intravenously administered monosaccharides.

2) to examine whether the previously reported differences in insulin response to intravenous and oral infusions of glucose is specific for glucose.

3) if this difference is not specific to glucose, is it related to:

a) structural differences in various monosaccharides?

b) the presence of a reducing group on the monosaccharide?

C. GENERAL APPROACH

The effects of intravenously administered sugars on serum insulin levels were studied first. This was done in order to establish whether the insulin response is specific to glucose-initiated hyperglycemia or not, and to compare their insulinotropic effects. A number of other sugars - mannose, fructose, galactose, ribose and xylose were also examined.

The above sugars were also administered orally and their effects on serum insulin levels were studied. Furthermore, the insulin responses to these two different routes of sugar administration were compared and the specificity of this additional mechanism was determined.

In order to determine if the process of insulin secretion is related to the active reducing group of a sugar moiety, the effects of intravenous and oral infusions of α-methyl glucoside on serum insulin levels were studied.

SECTION II

REVIEW OF LITERATURE

A. DISCOVERY, BIOSYNTHESIS, STORAGE AND SECRETION OF INSULIN

Since the discovery of insulin in 1922 by Banting and Best (4), the phenomenon of insulin secretion has been the object of extensive studies.

The histological studies of Lacy (5) among others have shown that insulin is synthesized from the individual amino acids at the endoplasmic reticulum of β-cells through the synthetic activity of ribosomes. It is then stored in membranous sacs located centrally in the β-cell. On stimulation by hyperglycemia or tolbutamide, these sacs solubilize and migrate outward and coalesce with the outer membrance. Ultimately a rupture takes place, discharging insulin granules into the extracellular space. Insulin granules then traverse to the pericapillary space through the microvilli formed by the outward projections of endoplasmic reticulum.

B. STIMULI FOR INSULIN SECRETION

1) Monosaccharides and related substances:

Various investigators have studied the phenomenon of insulin secretion in response to intravenous infusion of monosaccharides and certain metabolites. Although the experimental conditions differ considerably, it has been established that the infusion of glucose enhanced insulin secretion. However, there is no general agreement concerning the insulin response following the infusion of other sugars.

Pozza et al. (6) studied the effects of different sugars on insulin secretion by means of cross-circulation experiments with dogs using the extent of hypoglycemia produced as an index of insulin secretion. They found that glucose, galactose,

ribose and d-arabinose were capable of stimulating insulin secretion whereas fructose, mannose, xylose, 1-arabinose, 3-methyl glucose, glucosamine, galactoronic acid and saline were without such effect.

In 1964, Boda (7) studied insulin secretion in response to intravenously administered hexoses in sheep. It was noted that glucose and galactose brought forth a rise in serum insulin levels readily, whereas fructose provoked a delayed insulin response associated with a delayed rise in the blood glucose concentration.

Karam et al. (8) investigated insulin secretion after the intravenous administration of different sugars in man. It was found that glucose and mannose caused considerable rise in serum insulin levels whereas galactose was ineffective. Their observation differed from others in that mannose was a stronger stimulus than glucose for insulin secretion.

Grodsky et al. (9) and Sussman et al.(10) studied this phenomenon by perfusing the isolated rat pancreas with different sugars. It was observed (9) that glucose and mannose stimulated the release of insulin equally whereas fructose was less effective. Galactose, xylose, 1-arabinose, pyruvate and 2-deoxyglycose were ineffective in stimulating insulin secretion. Similar findings were reported by Sussman et al. (10).

Coore et al. (12) and Malaisse et al. (13) studied the release of insulin in vitro with rabbit and rat pancreas slices respectively. Coore et al. (12) reported that the presence of glucose and mannose in the incubation mixture, stimulated insulin release whereas all other sugars tested were ineffective. Malaisse et al. (13) found that glucose and mannose enhanced insulin secretion whereas fructose, galactose, ribose, xylose, arabinose and 2-deoxyglucose were

ineffective.

Goetz et al. (11) investigated the secretion of insulin by infusing different monosaccharides into the portal vein of anasthetized dogs. They found that glucose, galactose and ribose stimulated insulin secretion although there was no rise in the arterial blood glucose level. 2-deoxyglucose had very little effect.

Some of these investigators suggested that the stimulation of insulin secretion is not related to the chemical structure of glucose (6, 9) but rather related to the metabolism of the sugar infused (6 - 9, 12, 14).

Various investigators have studied the process of insulin secretion in relation to the different metabolic pathways. Their findings are briefly discussed as follows:

a) Sugar transport: Since in vitro studies of Coore et al. (12) have shown that the addition of phlorrhizin, an inhibitor of the cell membrane transport, to the incubation medium did not block the enhanced secretion of insulin by glucose, it was considered unlikely that the transport of glucose into a cell would provoke a stimulus for insulin secretion.

b) Phosphorylation of sugar: Coore et al. (12) and Malaisse et al.(13) have shown independently that mannoheptulose, when added to the incubation mixture containing pancreatic slices and glucose, completely abolished the insulinotropic effect of glucose. Based upon such findings, they suggested that phosphorylation of sugars may be related somehow to insulin secretion. Sussman et al.(10) concluded that since glucose, fructose and mannose stimulate the release of insulin immediately after their infusions and further more, since

these hexoses have been shown to be capable of being phosphorylated in the islets of toad fish, they extrapolated these results to suggest that the phosphorylation of these sugars in the islet cell may stimulate the secretion of insulin. This hypothesis was further supported by the findings of R-Candela (15) who showed that the addition of ATP to the medium containing slices of rabbit pancreas, produced more insulin than in the control experiments. They suggested that ATP has a role in insulin secretion.

c) Glycolysis: Since anoxia, an accelerator of glycolysis, did not augment the release of insulin, brought about by glucose (12, 13), it was concluded that the metabolism of glucose through glycolysis does not seem to be responsible for the stimulation of insulin secretion.

d) Hexose monophosphate shunt: Coore et al. (12) have shown that the presence of phenazine methosulphate in the incubation mixture had no effect on insulin secretion brought about by glucose. Since this substance has been shown to accelerate glucose oxidation through this pathway, it was concluded that the metabolism of glucose through the direct oxidative pathway is not associated with the phenomenon of insulin secretion, Kuzya et al. (16) have shown, however, that intravenous injection of xylitol stimulated the secretion of insulin more strongly than glucose. Since xylitol enters the direct oxidative pathway through D-xylulose-5-phosphate, they suggested that some common metabolic process shared by both xylitol and glucose might be intimately related to insulin secretion.

e) Metabolites: Coore et al. (12) have shown that glutamate, fumarate and pyruvate but not β-hydroxybutyrate, acetoacetate and citrate stimulated insulin release. The evidence so far is not conclusive about the

stimulatory effects of these metabolites and it is not known whether these substances penetrate into the cell or not.

f) Amino acids: Intravenous infusion of certain amino acids, such as arginine (20, 21) and leucine (20, 24, 25), has been shown to stimulate insulin secretion. The rise in blood amino acids has been considered to be a physiological stimulus for the release of insulin (20, 22). It was also noted that the infusion of amino acids raised the blood glucose concentration suggesting gluconeogenesis. Further studies of Rabinowitz et al. (21) have shown that epinephrine depressed glucose-initiated insulin release, whereas it failed to depress the arginine-initiated insulin secretion. Furthermore hyperglycemia produced by the infusion of arginine, did not block the secretion of growth hormone whereas glucose-produced hyperglycemis blocked the secretion of growth hormone. Based upon these observations, it was concluded that arginine, if not other amino acids, stimulates insulin secretion by some separate mechanism than glucose.

Intravenous infusion of leucine has been shown to cause hypoglycemia in man (23). Floyd et al. (24) have also shown that leucine produced hypoglycemia by elevating the plasma insulin levels in man. Recently, Floyd et al. (25) have reported that leucine enhanced the release of insulin from the pancreatic β-cells and thereby cause hypoglycemia.

2) Intestinal Hormones:

Scow et al. (26), Arnould et al. (27) and Dupre (28, 30) studied independently the rate of disappearance either of orally administered glucose in dogs, (26) or of glucose-C^{14}, injected prior to the gastric intubation of glucose in unanesthetized dogs (27), or of intravenously

infused glucose after the ingestion of glucose in man (28, 30). In all these studies, it was found that oral administration of glucose enhanced the rate of removal of glucose considerably and it was suggested that probably, greater amount of insulin was released after the oral infusion of glucose than after the intravenous administration of glucose.

McIntyre et al. (32, 33) and Elrick et al. (34) have shown independently that theinsulin response to oral glucose was much greater than to intravenous glucose despite the fact that the rise in blood glucose concentration after oral glucose was much smaller than after the intravenous infusion of glucose. Based on these observations, it was postulated that a humoral substance was released from the small intestine during the absorption of glucose which stimulated the release of insulin.

In contrast, Goetz et al. (11) have shown that the infusion of different monosaccharides into the portal vein of dogs, stimulated the release of insulin from the pancreas despite the fact that there was no rise in the arterial blood glucose concentration. They suggested the existence of a mechanism in the hepatoportal system which responds to the changes in sugar concentration of the portal blood and enhances the release of insulin by β-cells of the pancreas. However, McIntyre et al. (33) and Dupre et al. (30) have shown independently that the characteristic insulin response to oral glucose still persisted in the patients who had their liver excluded by portacaval shunts. They suggested that the perfusion of liver was not necessary for this insulin response to oral glucose. However, this does not explain the findings of Geotz et al. (11).

a) Secretin: Dupre et al. (28, 29) prepared extracts of crude

secretin from the hog duodenum and found that a rapid injection of these extracts with the second intravenous infusion of glucose in man, enhanced the rate of removal of intravenous glucose. Further studies (38) showed that secretin stimulates insulin secretion in man and is partly responsible for producing a greater insulin response to oral glucose. Moreover, they have reported recently (31) that simultaneous injection of gut mucosal extracts and glucose intravenously in man, produced insulin response which was similar to that observed following the oral glucose. However, this extract was claimed to be biologically distinguishable from either pancreozymin or secretin or glucagon.

In vitro studies of Pfieffer et al.(35) with the isolated pancreas of dogs and rabbits, have shown that the addition of secretin to the medium lacking glucose, stimulated insulin release by sixfold. It was concluded that secretin stimulates insulin secretion by the pancreas independent of glucose.

Similar conclusions were derived by Unger et al. (37) who injected secretin endoportally into anesthetized dogs and found a marked rise in the immunoreactive insulin in the pancreatico-duodenal vein blood, with no concomitant change in the arterial blood glucose concentration.

Since most of the preparations of secretin contained minute amount of glucagon which has been shown to stimulate insulin secretion(42, 44 - 47), it was of interest to investigate whether the stimulatory effect of secretin was in fact due to secretin rather than its contaminant, glucagon. Unger et al. (36) examined such a possibility by injecting crude and pure secretin or glucagon into the portal vein of dogs. They found an immediate rise (peak at 1-3 minutes

after the injection) in insulin concentration of pancreatico-duodenal vein blood with no change in the arterial blood glucose level after the infusion of both crude and pure secretin whereas a delayed insulin response (peak response at 3-6) minutes of injection) accompanied with a rise in the arterial blood glucose concentration followed the injection of glucagon. Moreover, pure secretin stimulated insulin secretion more strongly than crude secretin. Based upon these findings, it was concluded that secretin stimulates the release of insulin independent of glucagon.

However, Boyns et al. (39) produced secretin endogenously by instilling citric acid into the duodenum of man and studied its effect on insulin secretion. No effect on both blood glucose and plasma insulin levels was observed. They concluded that it seems unlikely that either secretin or pancreozymin has a role in enhancing insulin secretion following the ingestion of glucose.

b) Pancreozymin: Dupre (29) has shown that a rapid intravenous injection of pancreozymin with the second intravenous injection of glucose, did not lower the half life of intravenous glucose. Unger et al. (37) injected pancreozymin endoportally into dogs and found a sharp rise in the immunoreactive insulin as well as glucagon levels in the pancreatico-duodenal vein blood and furthermore, a concomitant rise in the arterial blood glucose concentration was noted. It was concluded that pancreozymin stimulates insulin secretion either by its direct effect on the β-cells of pancreas or indirectly by stimulating the secretion of glucagon by α-cells of the pancreas.

Meade et al. (40) supported the above conclusions by observing that

an intravenous injection of pancreozymin into the peripheral vein of dogs, brought forth a marked rise in insulin concentration of the portal blood which was accompanied by a slight rise in the arterial blood glucose and by a decrease in the plasma free fatty acids.

3) Other Hormones:

a) Glucagon: Samols et al. (42) investigated the effects of intravenously injected glucagon on plasma insulin and blood glucose levels in man. A marked rise in plasma insulin along with a slight rise in blood glucose concentration was observed. Furthermore, a greater insulin response was observed when glucagon was infused simultaneously with glucose. It was suggested from these findings that the absorption of ingested glucose may stimulate the secretion of glucagon from the gut and glucagon is probably the active factor which produces a better insulin response to oral glucose.

The above postulate was examined by Samols et al. (43) and Lawrence et al. (48) by measuring the circulating levels of both glucagon and insulin after oral and intravenous administration of glucose in men. In both these studies, a marked increase in both plasma glucagon as well as insulin level followed the ingestion of glucose whereas no such rise in glucagon accompanied the rise in insulin after intravenous glucose. Based upon these observations, it was concluded that the absorpotion of ingested glucose through the small intestine stimulates the release of glucagon from α-cells of the pancreas by a humoral or reflex mechanism. Since Unger et al. (41) have shown the presence of immunoassayable glucagon in the gastro-intestinal tract of rat, dog and human, the possibility of glucagon being secreted by the gut after oral

glucose, still remains open for future investigations.

Since glucagon is known to cause hyperglycemia, it was of interest to investigate whether glucagon stimulated insulin release by its direct effect on β-cells of the pancreas or indirectly by its hyperglycemic effect. Crockford et al. (44) examined such a possibility by producing hyperglycemia comparable to the one observed after the infusion of glucagon, by administering glucose intravenously in man. It was found that a much greater rise in serum insulin level occurred following the infusion of glucagon than after the administration of glucose. Furthermore, a second rapid injection of glucagon after the continuous and slow infusion of glucagon for 3 1/2 hours, produced a sharp rise in serum insulin level without concomitant change in the blood glucose concentration. Based upon these findings, it was concluded that glucagon stimulates insulin secretion directly and independent of its hyperglycemic effect.

Campbell et al. (45) have also shown that the intravenous injection of glucagon in dogs, increased the insulin/glucose ratio significantly. However, this ratio remained constant after the intravenous infusion of glucose. They suggested that this rise in insulin/glucose ratio indicates the presence of an additional stimulus besides hyperglycemia to the pancreas.

In vitro studies of Turner et al. (46) with slices of rabbit pancreas, showed that glucagon when added in high concentration (5.0 μg/ml.), augmented the insulin response provoked by low and high glucose concentrations in the incubation medium. However, the addition of glucagon at low concentration (0.5 μg/ml.) did not potentiate insulin release at low concentrations of

glucose whereas it augmented the release of insulin by high concentration of glucose in the incubation medium.

Vecchio et al. (47) studied the effects of glucagon on insulin secretion with tissue cultures and found a clear cut and dose-dependent insulin response to the addition of glucagon. It was concluded that glucagon stimulates insulin secretion directly from the explants.

b) Gastrin: Gastrin when injected endoportally into dogs (37), has been shown to stimulate insulin secretion without affecting the blood glucose and plasma glucagon concentration.

4) Nervous Stimulus:

a) Stimulation of vagus nerve: Recent findings of Daniel et al. (52) and of Kaneto et al. (53) have shown that electrical stimulation of the vagus nerve in baboon and dog respectively enhanced the release of immunoreactive insulin. Based upon this evidence they suggested a neuroendocrine mechanism which regulates insulin secretion. However, it has been shown (78) that a pancreatic graft in the neck of a dog responded equally well to the rise in blood glucose concentration despite the fact that its nervous supply has been disrupted. The perfusion studies of pancreas have also shown the insignificance of the nervous system in the regulation of insulin secretion.

C. INHIBITORS OF INSULIN SECRETION

1) Epinephrine: Epinephrine has been shown to inhibit the release of insulin from the pancreas (49-51). Furthermore, intravenous injection of epinephrine simultaneously with either glucose or glucagon or tolbutamide, has been shown (49) to abolish the stimulatory effects of these substances.

Since epinephrine mobilized the release of free fatty acids, it was of an interest to establish whether epinephrine inhibited insulin secretion by its lipolytic action or by its direct effect at the β-cell level. Porte et al. (50, 51) investigated such a possibility by injecting nicotinic acid along with epinephrine in man. It was found that epinephrine retained its inhibitory effect despite the lack of rise in plasma free fatty acids. Based on these findings, it was concluded that epinephrine blocks insulin secretion by its direct effect on the β-cells.

The mechanism by which epinephrine inhibits the release of insulin is not fully understood. However, further studies of Porte (50, 51) have shown that α-and β-adrenergic receptors are involved in the inhibition of insulin secretion by epinephrine. They have suggested that probably, epinephrine stimulates β-adrenergic receptors which in turn stimulate the secretion of insulin but at the same time, it enhances the inhibition of insulin secretion by α-adrenergic receptors, thereby maintaining the basal serum insulin levels.

2) Norepinephrine: Intravenous injection of norepinephrine in man has been shown to inhibit secretion. However, it was found to be less potent in its inhibitory effect than epinephrine (49).

SECTION III

MATERIALS, EXPERIMENTAL PROCEDURES AND METHODOLOGY

A. MATERIALS

1) Animals: Healthy rabbits of both sexes, of mixed strains and weighing from 3 - 4 kilograms were used. These animals were kept in a ventilated room at 27°C in individual cages and were fed with rabbit chow. Cabbage and carrots were given on every other day.

2) Anesthesia: The animals were anesthetized by an intravenous injection of sodium pentobarbital (British Drug House) solution. A dose of thirty milligrams per kilogram body weight of animal, in a final volume of 4 mls. made with the physiological saline solution, was used.

3) Test substances and dosage used: A solution of test substance was prepared fresh on the day of its use in warm distilled water. During the tests of sugars, the dosage used was 1.5 grams contained in a volume of three millilitres per kilogram body weight of animal. i.e. 1.5 grams/3cc./Kg.b.w. All the sugars tested were of reagent grades and were purchased from Fisher Scientific Corporation. During the tests with amino acids, equimoles of amino acids to that of hexose dose, were injected.

4) Control Experiments: Equal volume of physiological saline solution (0.85% sodium chloride) was injected.

B. EXPERIMENTAL PROCEDURES

1) Design of Experiments: Both the animal used and the substance to be infused into that particular animal were selected in a completely random manner. Some of the animals were used more than once in this study. However, they were

never injected twice with the same substance by the same route.

2) Fasting and Anesthesia: The animals were fasted for 8 - 10 hours overnight and water was given during these hours. Next morning, the animal was weighed, placed in a bleeding box and anesthetized with an intravenous injection of sodium pentobarbital.

The pentobarbital was injected according to the response of the animal. Usually the total volume of solution was given in two injections. Two mls. were first injected and the behaviour of the animal was observed. If the animal went into deep anesthetic state, no further injection was given. However, if the animal was still active, a second injection of 2 mls. or less was given. By this procedure, the level of anesthia was made approximately the same in all the animals.

A basal (0') blood sample was taken by a cardiac puncture. Subsequent blood samples were also withdrawn by the same technique.

3) Intravenous tests: The test substance was injected rapidly into the marginal ear vein of the animal. Blood samples of 2-3 mls. each were withdrawn at 5, 15, 30, 60 and 120 minutes after the injection. Pyruvate was found to be toxic when injected rapidly and therefore was infused slowly over 5 minutes after adjusting its pH to 7.40.

4) Oral tests: The test substance was administered by stomach tube and the solution remaining in the tube was flushed with 5 mls. of distilled water and the blood samples were withdrawn at regular time intervals.

5) Control tests: Equal volumes of physiological saline solution as of other test substances, were injected either intravenously or orally into

animals and the blood samples were taken at the same intervals of time and by usual technique.

C. METHODS OF ANALYSIS

1) Blood sugars analysis: Each blood sample was analysed for the total reducing activity by the method of Nelson and Somogyi (57) and for true glucose concentration by Glucose Oxidase Method (58). Appropriate standards were run along with each set of determination.

The amount of actual sugar, other than glucose, present in the blood sample was determined by substracting true glucose concentration from that of total reducing sugars.

In experiments where α-methyl glucoside was tested, true glucose concentration was estimated by glucose oxidase method but in order to determine total reducing activity, $Ba(OH)_2$-zinc sulphate filterate was hydrolysed in 1 N HCl for two hours at $100^{o}C$. The hydrolysate was cooled and neutralized with sodium hydroxide solution and was then analysed for the total reducing activity by Nelson and Somogyi method (57).

2) Assay of Insulin: Serum insulin concentrations were determined by the modifications of immunoassay procedures of Soeldner and Slone (59) and of Meade et al. (60). Details of this modified immunoassay are given in the appendix. The recovery of added insulin with this immunoassay procedure, was in the range of seventy to eighty percent.

D. PARAMETERS

1) Blood sugar concentration was expressed as milligrams percent (mgs. %).

2) Serum insulin concentration (S.I.L.) was expressed in relative terms taking each animal's basal (0') level as 1.0. This way of expressing serum insulin concentration eliminates the considerable variation in the basal insulin levels that can occur i.e. 2-25 μUnits/ml., among animals.

E. PRESENTATION OF RESULTS

The changes in blood levels of monosaccharides, true glucose and serum insulin concentration resulting from either intravenous or oral administration of sugars, are shown in respective histograms where these three parameters are represented by three bars at each interval of time. Each bar represents the mean value plus or minus standard error ($\bar{X} \pm$S.E.). The inset of histograms shows a semilogarithmic plot of actual blood sugar level (mgs %) and time in minutes after the infusion.

Left ordinate of each histogram represents actual blood sugar and true blood glucose concentrations (mgs %) and the right ordinate represents serum insulin levels (Insulin x basal insulin level). The tables containing the results in details are given in the appendix.

F. STATISTICAL ANALYSIS

In the control saline infused animals, an analysis of variance (single classification) showed that the means of serum insulin levels at six time intervals (0, 5, 15, 30, 60 and 120 minutes) did not differ significantly from one another. Likewise, the means of true blood glucose levels at the same time intervals did not differ significantly from one another.

The details of these statistics are shown in Appendix B. As there was no significant difference between the mean insulin level at any time following

saline infusions, an overall means was obtained by averaging the six means of serum insulin levels at 0, 5, 15, 30, 60 and 120 minutes after infusions.

The means serum insulin level at each time interval after infusing the test substance, was compared with the overall mean value. The significance of the differences (p) was determined by a t-test programmed for unequal, unpaired and small number of observations.

SECTION IV

RESULTS

A. EFFECTS OF INTRAVENOUSLY ADMINISTERED MONOSACCHARIDES AND OTHER SUBSTANCES ON SERUM INSULIN AND BLOOD GLUCOSE LEVELS.

1) Saline: Intravenous injection of saline in volume equal to that used during the infusion of sugars i.e. 3 cc's of saline/kg. b.wt., did not change the serum insulin level nor the blood sugar level significantly ($p^* > .05$)[1]. These results are shown in Figure 1 and Table 1.

2) Glucose: Intravenous glucose stimulated the release on insulin and the results of these experiments are illustrated in Figure 2 and Table II. A sustained insulin response followed the intravenous infusion of glucose as is evident from the serum insulin levels which were well above the basal level ($p < .05$) except at 120 minute time interval, despite the fact that the blood glucose concentration declined exponentially. The maximum rise in serum insulin level was from 5 to 15 minutes after the injection. Furthermore, it should be noted that the increase in serum insulin levels was not directly related to the amount of glucose present in the blood stream.

3) Mannose: Intravenous infusion of mannose stimulated insulin secretion considerably (Figure 3 and Table III) and the maximum rise in serum insulin level i.e. 3.85 ± 0.87 ($p < .01$) occurred at 5 minutes. However, unlike glucose, the insulin response was very transient and the serum insulin

---

1 The significance of difference was determined by the analysis of variance. The details of this analysis are given in Appendix B.

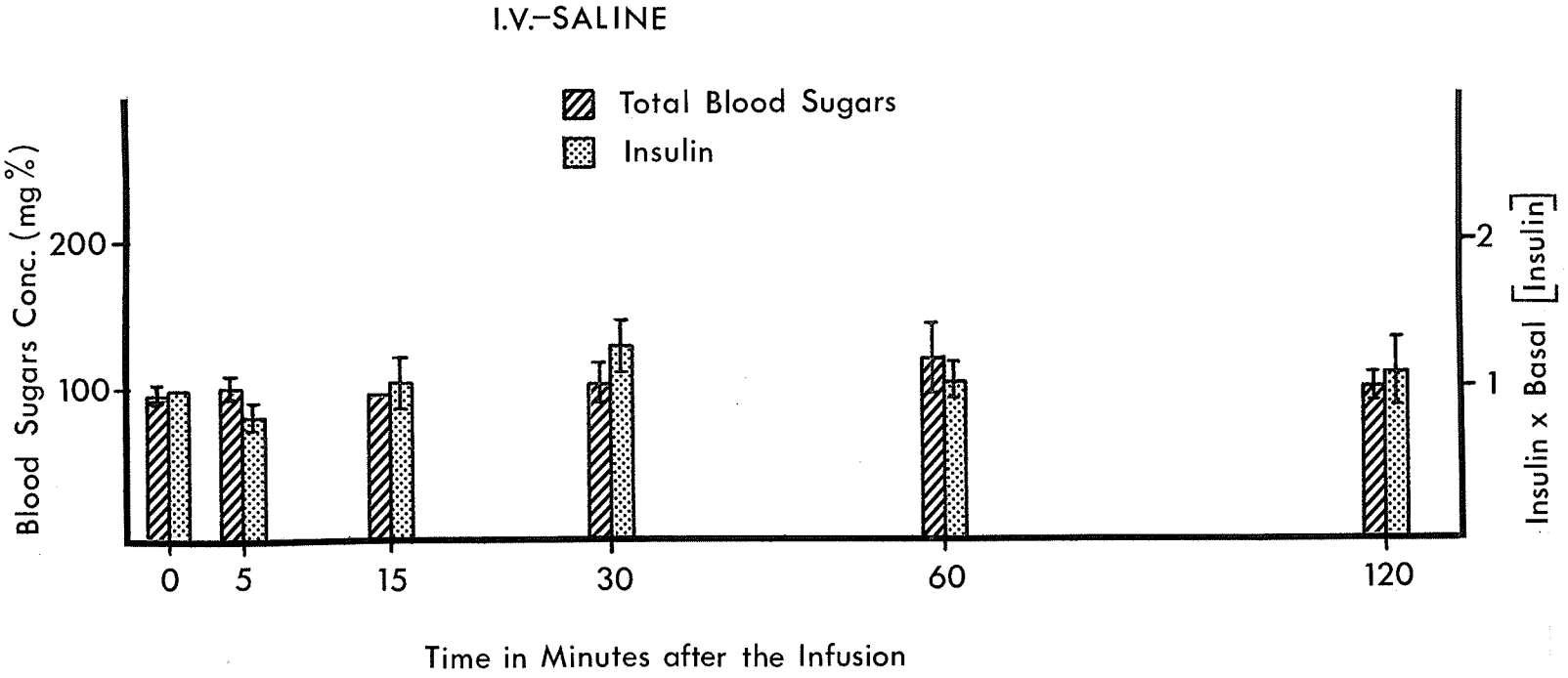

Figure 1: Effects of rapid intravenous infusion of saline on blood sugar and serum insulin levels.

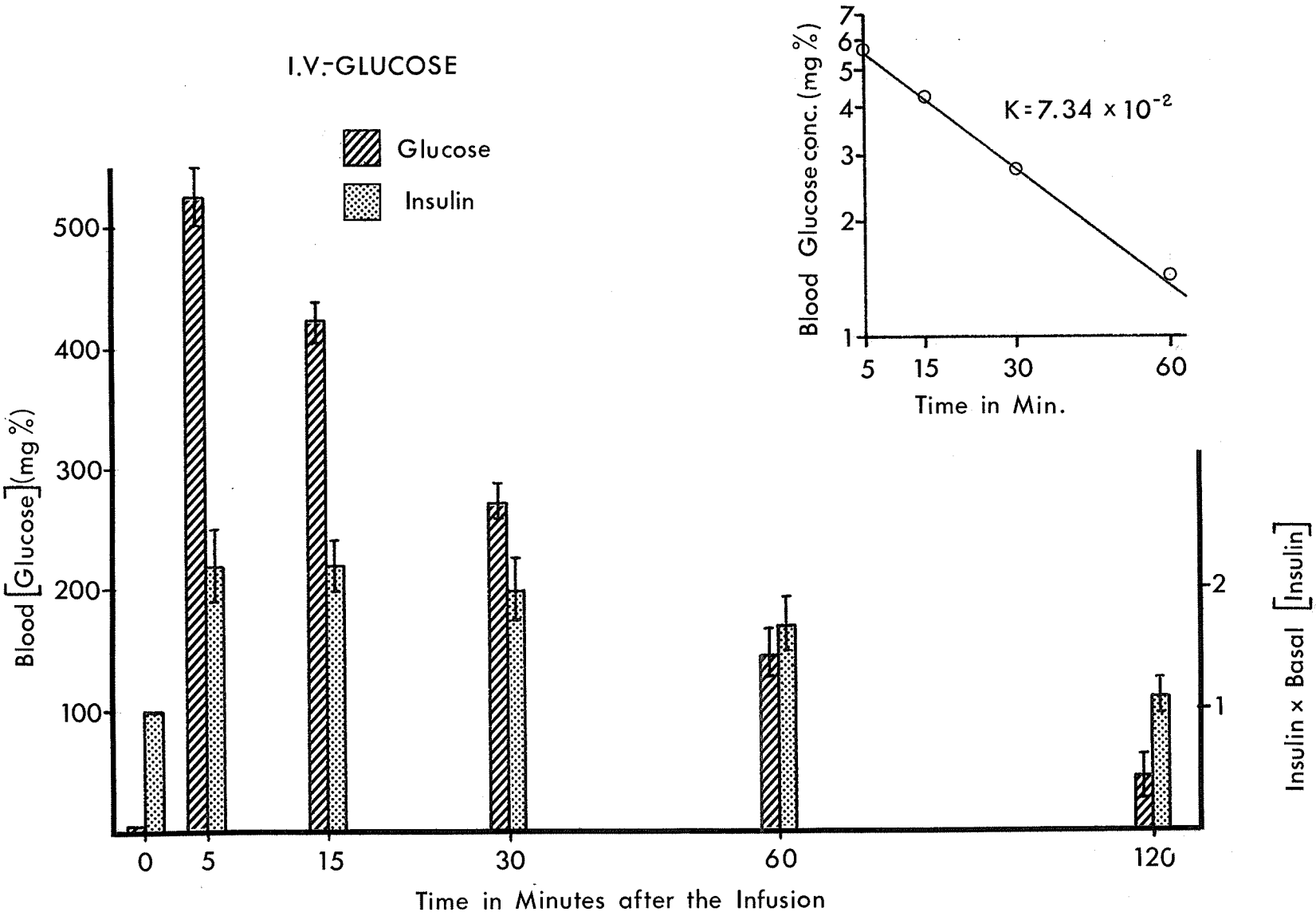

Figure 2: Effects of rapid intravenous infusion of D(+)glucose on blood glucose and serum insulin levels.
Inplot. Shows a semilogarithmic plot of blood glucose levels versus time in minutes after the infusion

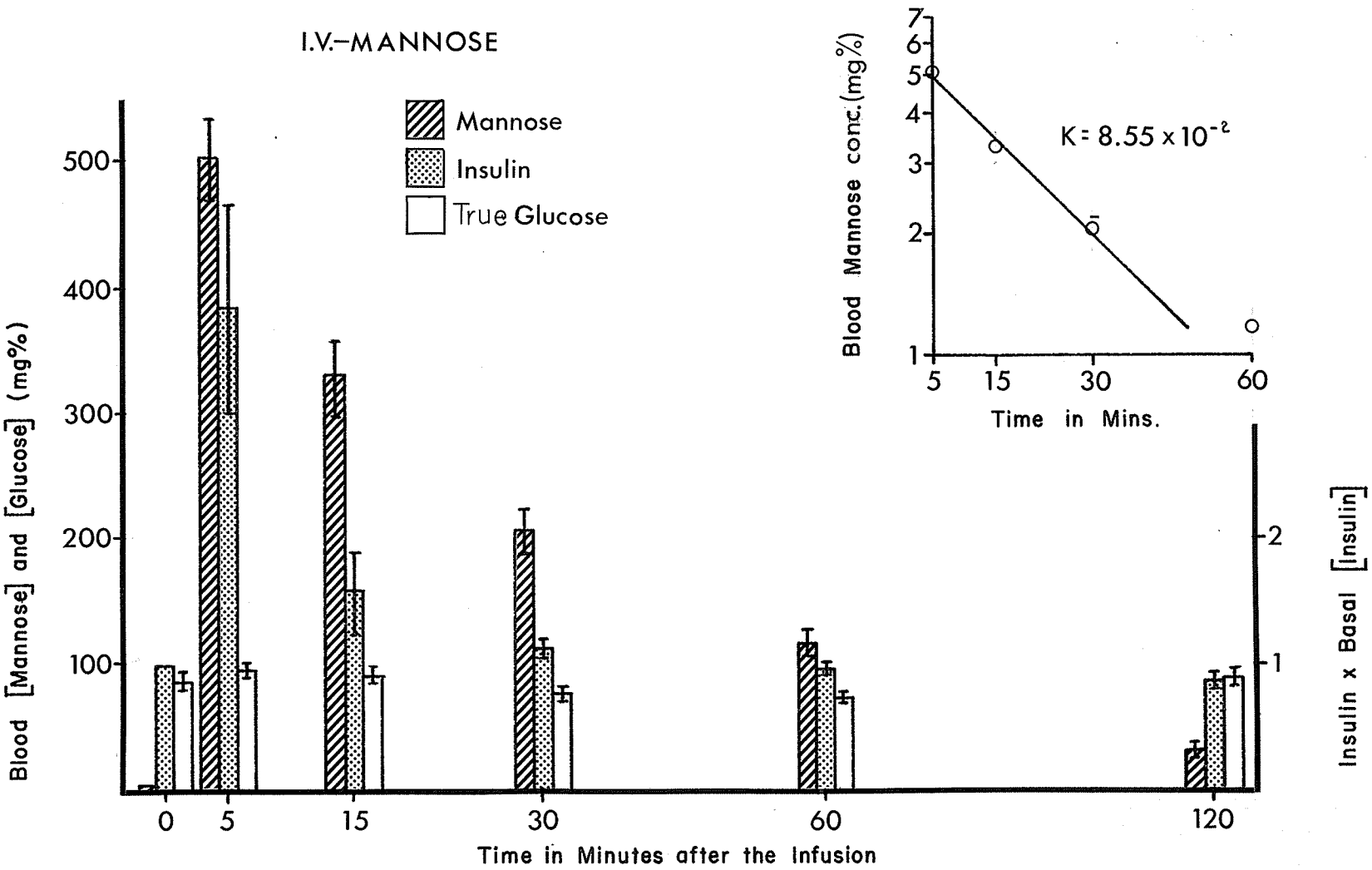

Figure 3: Effects of rapid intravenous infusion of D(+) mannose on true blood glucose, blood mannose and serum insulin levels.
Inplot. Shows a semilogarithmic plot of blood mannose concentration

levels dropped to almost basal level at 15 minutes ($p < .20$) after the infusion and below basal level at 60 and 120 minute time intervals. No significant changes in the true blood glucose concentrations ($p^* \geq .05$)[1] were observed following the infusion of mannose.

4) Fructose: Figure 4 and Table IV illustrate the effects of intravenous fructose on serum insulin and on blood glucose concentrations. It can be seen that serum insulin level increased promptly to a peak level of 2.85 ± 0.09 fold ($p < .001$) at five minutes and remained well above the basal level for one hour after its infusion. The insulin response to intravenous fructose was somewhat similar to that following intravenous glucose and was dissimilar to that after intravenous mannose. The maximum rise in blood fructose concentration (470 ± 29 mgs %) following its injection was somewhat lower ($p < .3$) than the mean maximum rise in blood sugar concentration (507 ± 8 mgs%) following the infusion of other monosaccharides. True blood glucose level did not change significantly ($p^* > .05$).

5) Ribose: The results of experiments where ribose was infused intravenously are shown in Figure 5 and Table V. Serum insulin levels increased immediately following the infusion and continued to rise to a maximum level of 1.70 ± 0.08 ($p < .001$) at 30 minutes and remained elevated thereafter. The insulin response to intravenous ribose was somewhat similar to glucose ($p < .10$) and quite similar to xylose ($p < .70$) but was less than mannose ($p < .05$) and fructose ($p < .001$) (Table XI, Figure 11). No significant changes ($p^* > .05$) in true blood

---

1. Details of statistical analysis are given in Appendix B.

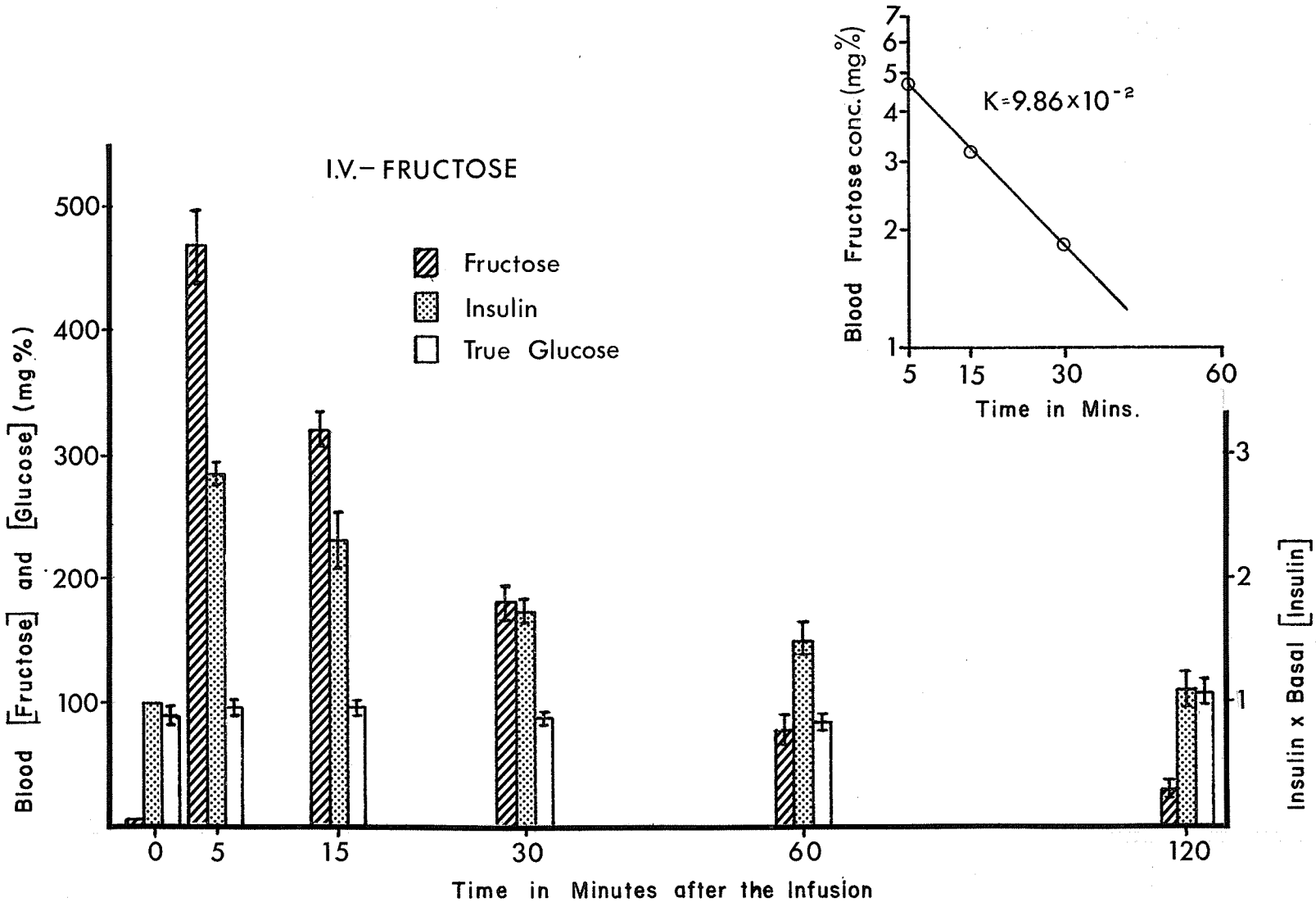

Figure 4: Effects of rapid intravenous infusion of D-fructose on true blood glucose, blood fructose and serum insulin levels.
Inplot. Shows a semilogarithmic plot of blood fructose levels versus time in minutes after the infusion.

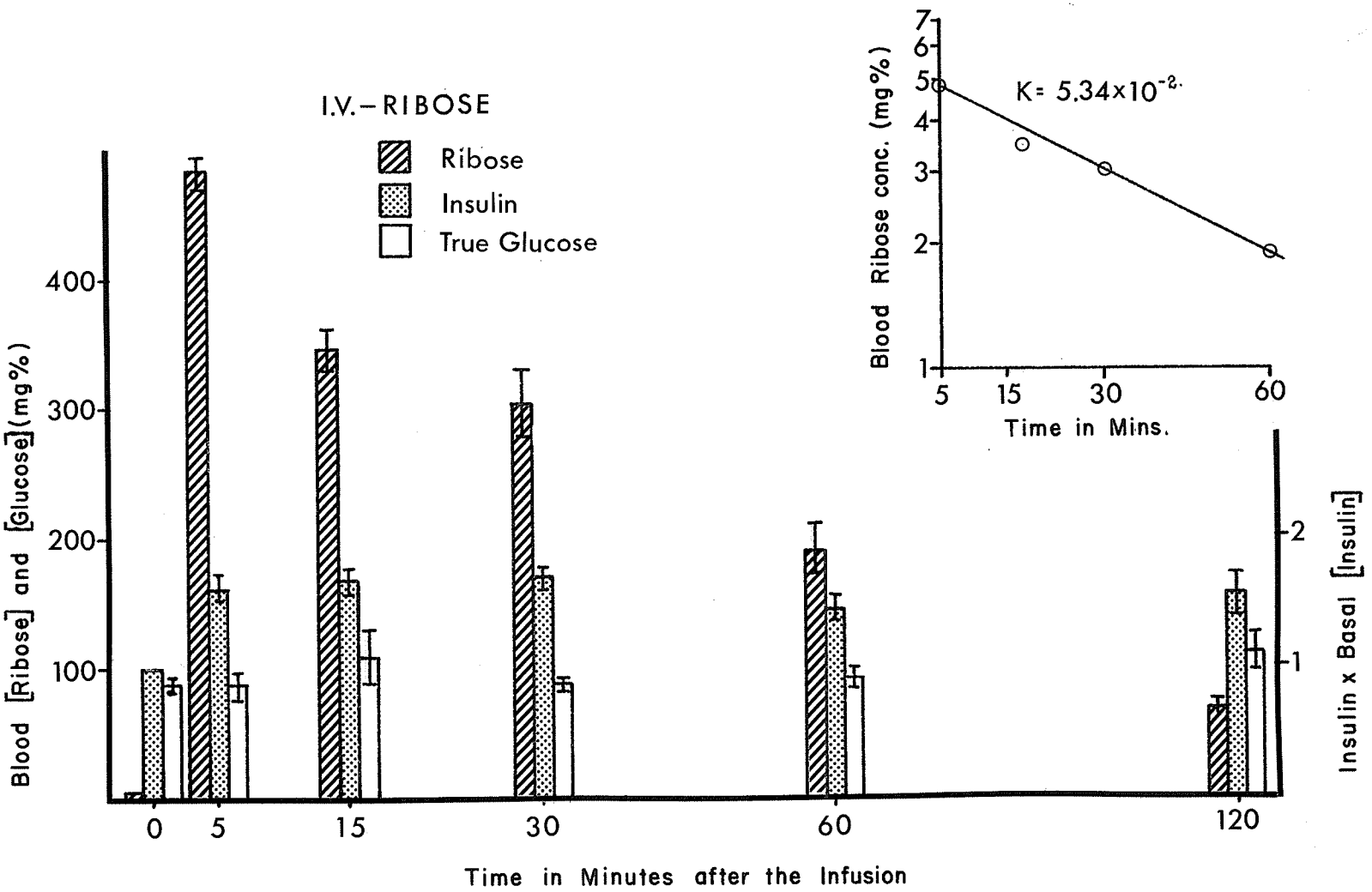

Figure 5: Effects of rapid intravenous infusion of D(-)ribose on true blood glucose, blood ribose and serum insulin levels.
Inplot. Shows a semilogarithmic plot of blood ribose concentration at different time intervals after the infusion.

glucose concentration were observed following the intravenous infusion of ribose.

6) Galactose: Figure 6 and Table VI depict the effects of intravenous galactose on serum insulin and true blood glucose concentrations. It is apparent that sudden rise in blood galactose level stimulated the release of insulin promptly reaching a peak level of 1.68 ± 0.14 ($p < .005$) at 5 minutes and serum insulin levels remained elevated until 30 minutes of infusion and dropped slightly thereafter. Insulin response was moderate and similar to the one observed following the infusion of glucose ($p < .10$), xylose ($p < .70$), ribose ($p < .90$) and pyruvate ($p < .90$). However, it was less than mannose ($p < .025$) and fructose ($p < .001$) (see Table XI and Figure 11 for comparison). A decrease of 0.3 fold ($p < .20$) in serum insulin level soon after the peak level at 5 minutes should be noted. Serum insulin increased again at 30 minutes and remained elevated thereafter. This decrease in serum insulin level at 5 minutes was associated with the least removal of galactose i.e. 17 mgs % during 15 - 30 minutes. True blood glucose concentration did not change very much ($p^* > .05$) during 2 hours of observations.

7) Xylose: Figure 7 and Table VII show the results obtained following the infusion of xylose intravenously. Serum insulin levels increased gradually to a peak level of 1.81 ± 0.21 ($p < .01$), 30 minutes after the injection, dropping to almost basal level ($p < .90$) at 60 minutes and showed a tendency to rise again at 120 minutes. This latter rise in serum insulin level was accompanied with a slight rise in true blood glucose levels i.e. from 84 mgs % at 60 minutes to 108 mgs % at 120 minutes. True blood glucose concentration did not change significantly ($p^* > .05$) during the course of experiments.

8) α-methyl Glucoside: α-methyl glucoside was infused intra-

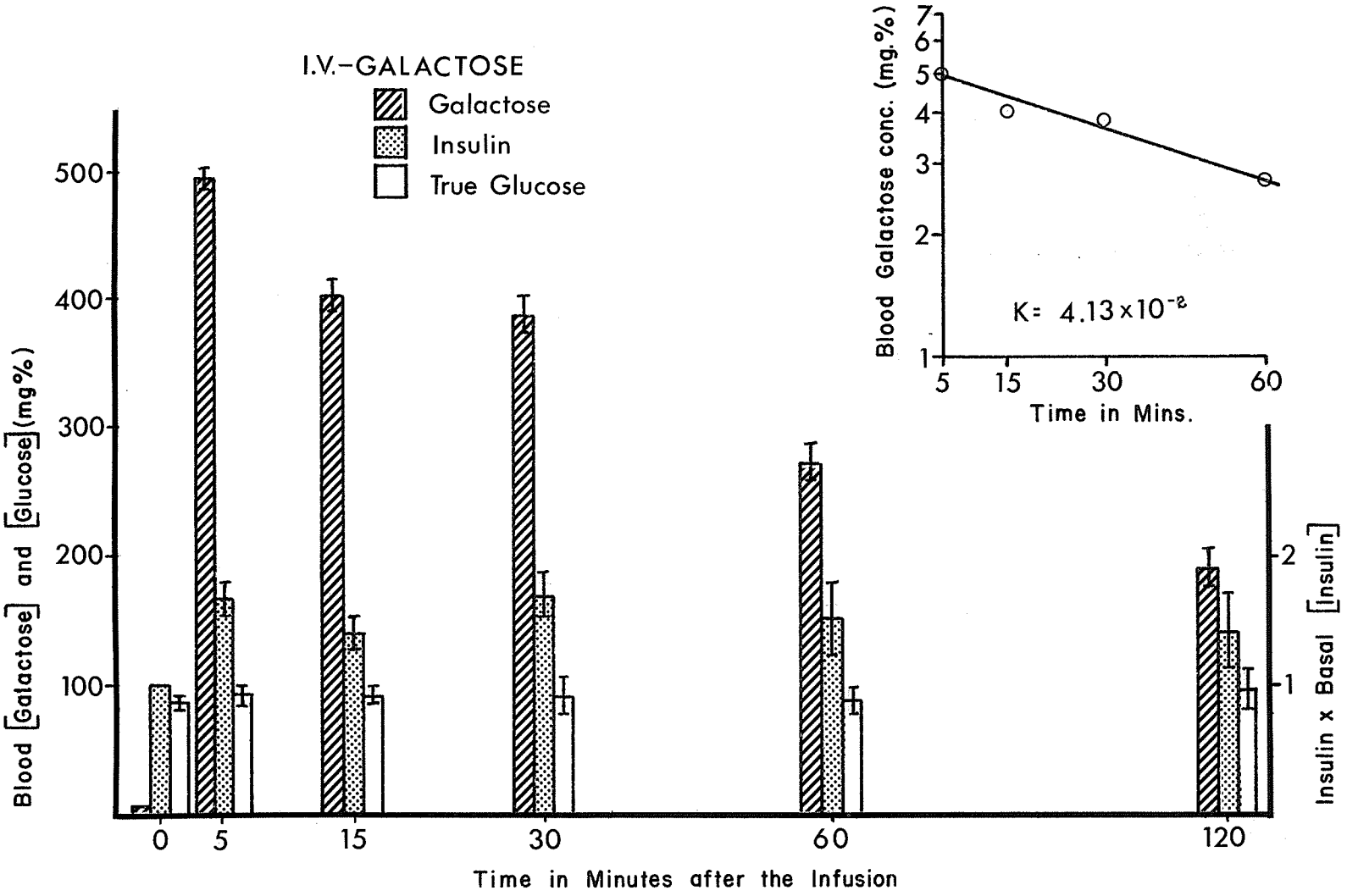

Figure 6: Effects of rapid intravenous infusion of D(+)galactose on true blood glucose, blood galactose and serum insulin levels.
Inplot. Shows the rate of removal of intravenous galactose.

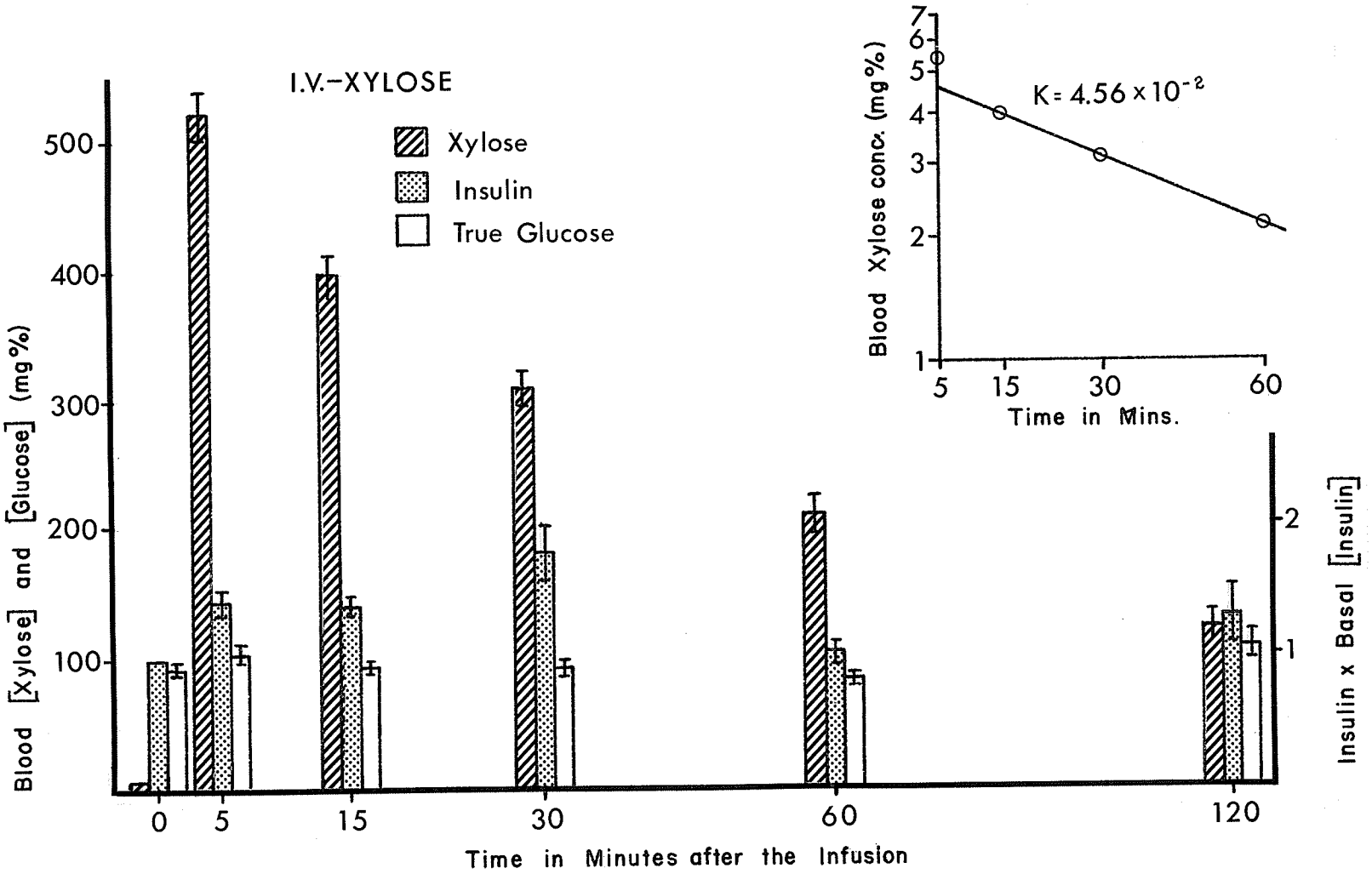

Figure 7: Effects of rapid intravenous infusion of D-Xylose on true blood glucose, blood xylose and serum insulin levels.
Inplot. Shows a semilogarithmic plot of blood xylose levels versus time in minutes after the infusion.

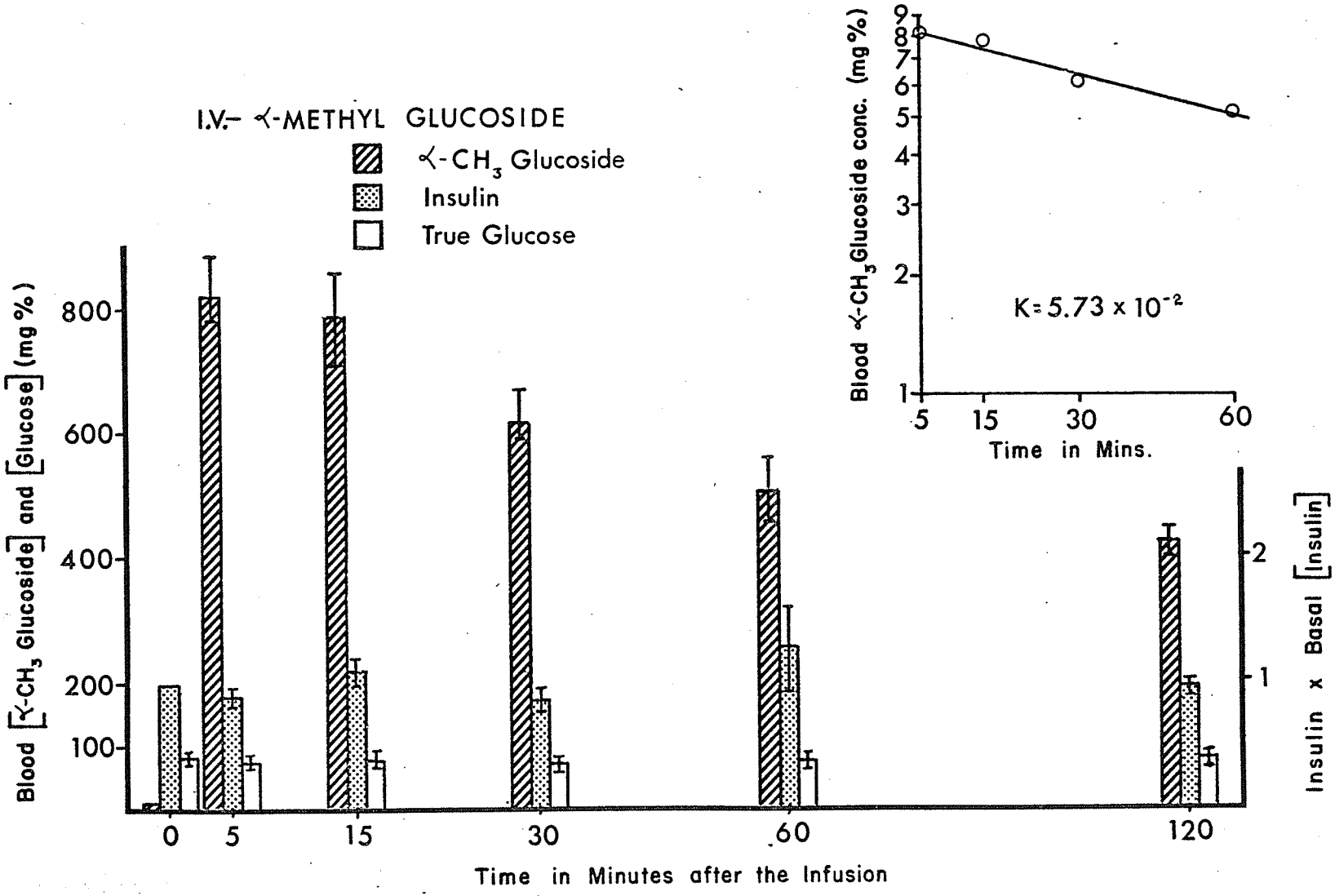

Figure 8: Effects of rapid intravenous infusion of α-methyl glucoside on true blood glucose, blood α-methyl glucoside and serum insulin levels.
Inplot. Shows the rate of removal of intravenous α-methyl glucoside.

venously to study its effects on both the true blood glucose and serum insulin levels. The results of these experiments are illustrated in Figure 8 and are listed in Table VIII in the appendix. Intravenous α-methyl glucoside did not stimulate the secretion of insulin since serum insulin level remained unchanged i.e. p was never less than 0.7. There was no significant change in true blood glucose concentration ($p^* > .05$) during the course of experiments.

9) Pyruvate: Figure 9 and Table IX illustrate the results of experiments where equimoles (0.91 gms/3 cc's/Kg.b.wt.) of pyruvate were infused intravenously. Serum insulin level increased gradually to a peak level of 1.63 ± 0.20 fold ($p < .05$), 60 minutes after the injection and declined thereafter. However, one should note that serum insulin levels at 30 and 60 minutes were the only ones which were significantly different from the mean control insulin level i.e. p less than 0.05 and 0.025 respectively. True blood glucose level did not change significantly ($p^* > .05$). Whether the infused pyruvate remained extracellular or penetrated into the cell is not certain. However, it has been shown that β-cells of the pancreas are as freely permeable to sugars as the liver cells and presumably pyruvate was intracellular.

B. COMPARISON OF INSULIN RESPONSES TO INTRAVENOUS MONOSACCHARIDES:

1) Mannose and Fructose: Insulin responses to intravenous mannose and fructose were similar ($p < .30$) (see Table XI and Figure 11 for comparison of insulin responses). The maximum rise in serum insulin levels occurred at five minutes after the infusion of fructose and mannose. However, these insulin responses differed in that the serum insulin level after fructose infusion, remained

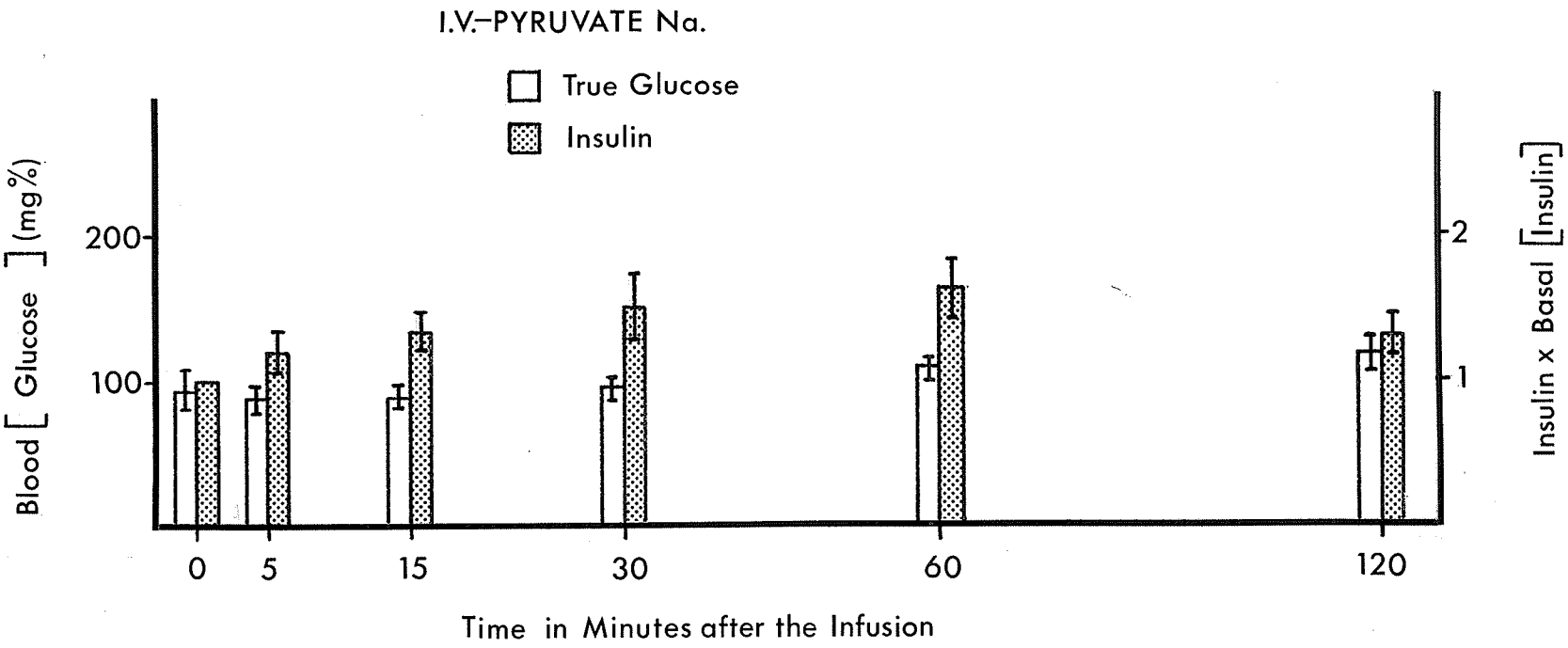

Figure 9: Effects of slow intravenous infusion of pyruvate (Equimoles to monosaccharides) on true blood glucose, and serum insulin levels.

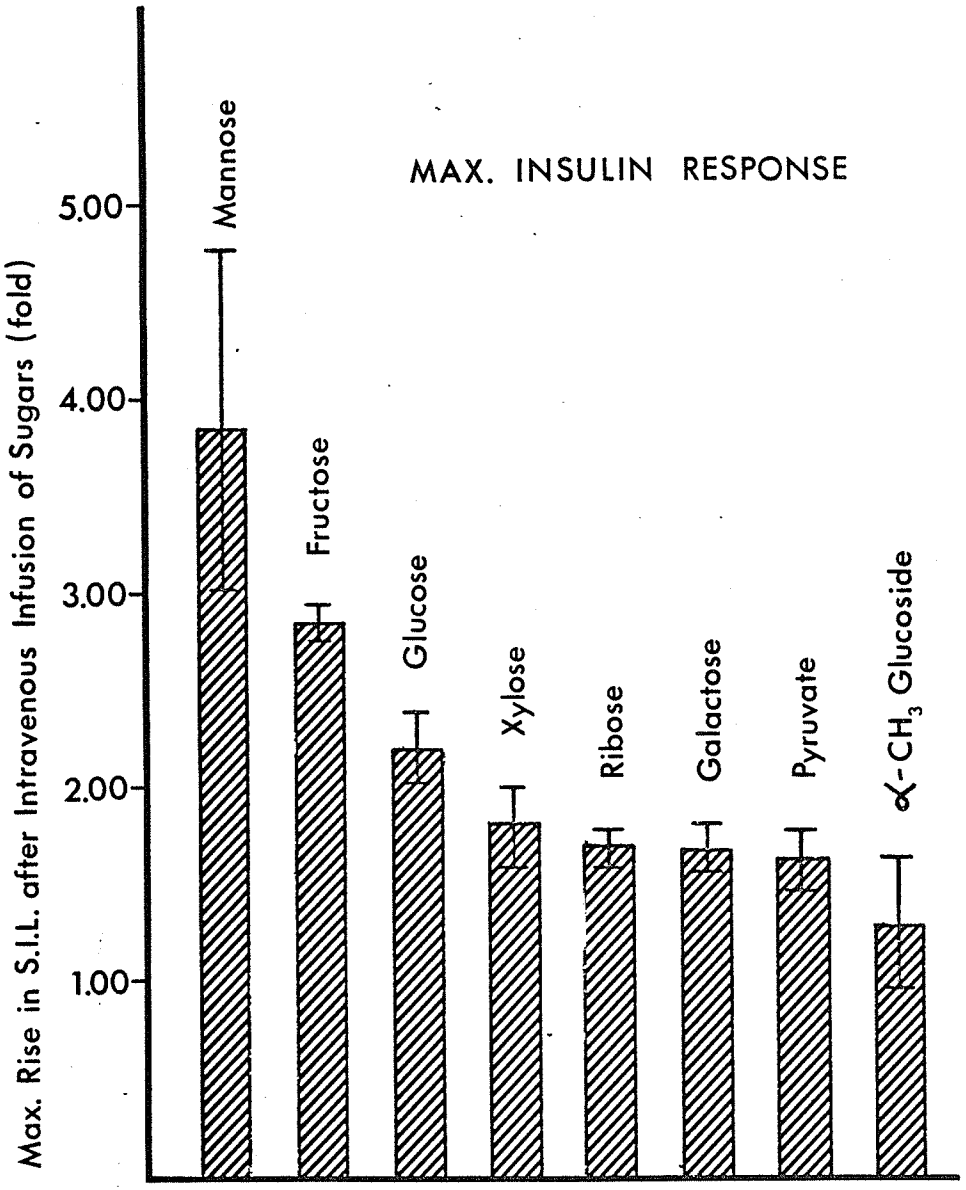

Figure 11: Shows the maximum rises in serum insulin levels following the infusion of various monosaccharides.

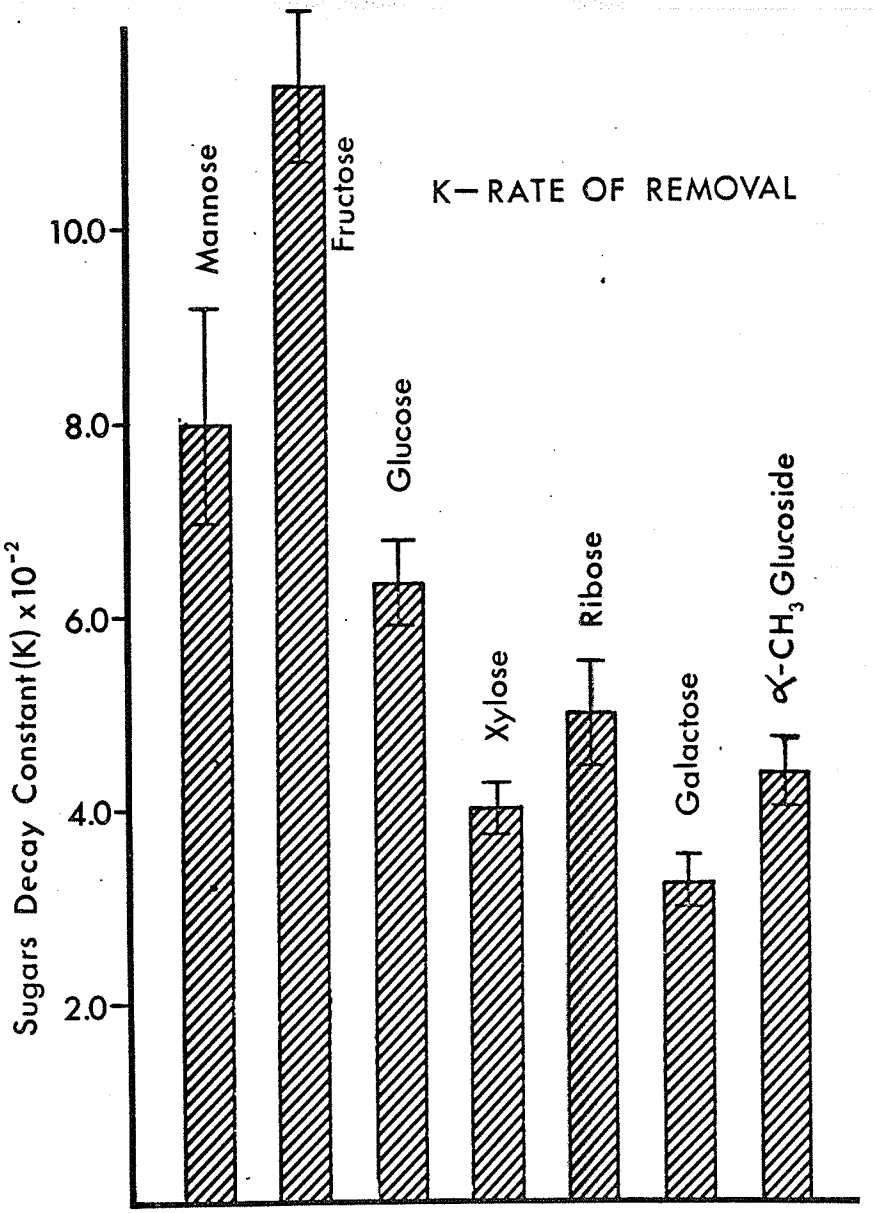

Figure 10: Shows the rates of removal (K's) of various monosaccharides infused intravenously.

well above the mean control insulin level for at least one hour after the infusion, whereas following the infusion of mannose, serum insulin level dropped to basal level at 15 minutes and below basal level at 30 and 60 minutes. Moreover, fructose disappeared from the blood stream at a little faster rate ($p < .05$) than mannose. Table X gives the comparison of rates of removal.

2) Glucose: Intravenous glucose brought forth an insulin response somewhat similar ($p < .10$) to that observed following intravenous mannose but was less effective ($p < .02$) than fructose (Table XI, Figure 11). However, unlike fructose, the maximum rise in serum insulin level was extended over 5-15 minutes of infusion. Furthermore, serum insulin levels were well above the basal insulin level for at least one hour after the infusion. Moreover, glucose was removed at a rate similar ($p < .10$) to mannose but was much slower ($p < .001$) than fructose (Table X, Figure 10).

Glucose, fructose and mannose produced marked and immediate insulin responses and were removed from the blood stream faster than other sugars i.e. xylose, ribose, α-methyl glucoside and galactose.

3) Galactose: Insulin response to intravenous galactose was moderate ($p < .005$) (Table XI, Figure 11) but somewhat similar ($p < .10$) to that after intravenous glucose in that the maximum rise in serum insulin level, occurred at 5 and 30 minutes with a slight decrease at 15 minutes. Serum insulin response was sustained over the remainder of time of observations. Of all the monosaccharides test, galactose was removed from the blood stream at the slowest rate (p was always less than 0.02) (Table X, Figure 10).

4) Xylose and Ribose: Insulin response to intravenous xylose ($p < .01$)

and ribose ($p < .001$) were moderate and were quite similar ($p < .70$) (Table XI, Figure 11). Serum insulin levels increased immediately following the injection of ribose and xylose and continued to rise to a peak level at 30 minutes. Insulin responses to both sugars were sustained over the first one hour at least. Both these sugars were removed at a similar ($p < .10$) rate, which was slower than fructose, glucose and mannose but similar to α-methyl glucoside and faster than galactose (Table X, Figure 10).

5) Pyruvate: Intravenous administration of pyruvate stimulated insulin secretion ($p < .025$), the least of all the substances investigated and serum insulin levels at 30 and 60 minute time intervals were the only ones which gained statistically significant differences from the mean control insulin level.

6) α-methyl Glucoside: Intravenous α-methyl glucoside did not stimulate insulin secretion. It was removed at a rate similar to xylose, ribose, and slightly faster than galactose (Table X, Figure 10).

Of all the sugars investigated with the exception of mannose, the rise in serum insulin level was not proportional to the amount of sugar present in the circulation.

C. EFFECTS OF ORALLY INFUSED MONOSACCHARIDES AND OTHER SUBSTANCES ON SERUM INSULIN AND BLOOD GLUCOSE LEVELS.

The purpose of investigating the effects of orally infused monosaccharides on serum insulin and blood glucose levels and the previous findings pertaining to such investigations, were cited under the purpose of the study and the review of the literature respectively. Suffice to state here

that the present experiments were carried out to elucidate the specificity of the mechanism which has been claimed to be responsible for a greater insulin response to oral glucose than to intravenous glucose.

1) Preliminary experiments: Since the mechanism whose specificity was to be investigated, has been suggested to be in the small intestine (33-34), it was considered desirable to inject glucose and other substances directly into the small intestine rather than intragastrically. This was achieved by exposing the small intestine by a cranio-caudal incision along the mid-ventral line. The results of such experiments are shown in diagram 12 and Table XII.

Serum insulin levels following intraduodenal infusion of glucose were compared with those found after the oral administration of glucose. Blood sugar levels following the infusion of glucose by both routes were also compared. It is quite apparent that the blood sugar levels were similar after the infusion of glucose by both routes i.e. p was never less than 0.3. Serum insulin levels, unlike those following oral glucose, fell below the basal level reaching a nadir ($p<.001$) at 30 minutes after the intraduodenal administration of glucose and seemed to start rising thereafter.

The data indicate that: 1) the stomach empties the sugar solution quite rapidly. 2) operative trauma inhibits the release of insulin. The latter findings are consistent with the findings of Allison et al. (80). Presumably, the operative stress stimulated the release of catecholamines which have been shown to inhibit the release of insulin. (49, 50).

In all the experiments to be reported hereafter, the test substance

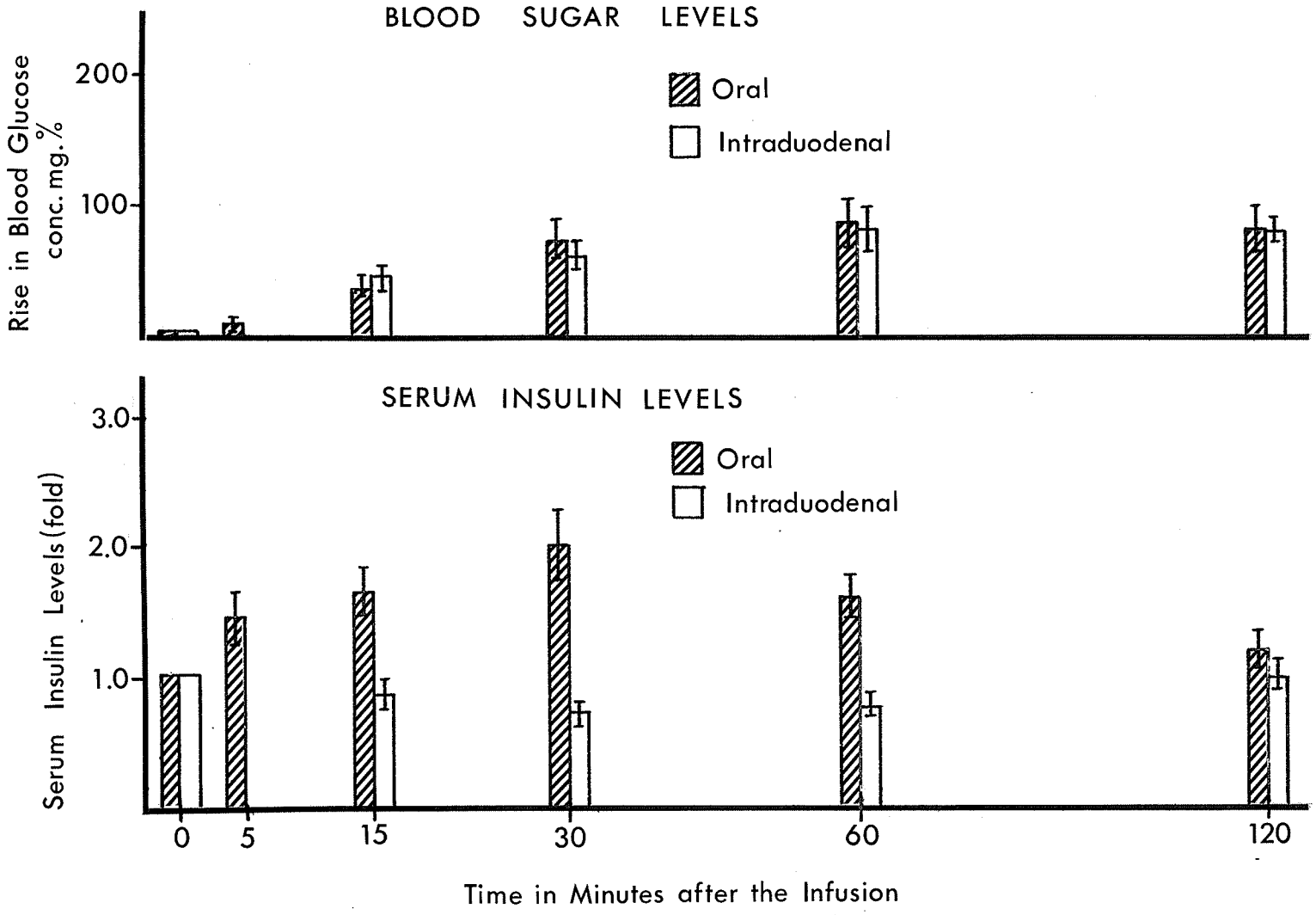

Figure 12: Effects of glucose infused intraduodenally and orally on the blood sugar and serum insulin levels.

was infused rapidly into the stomach as explained earlier in the experimental procedures.

2) Saline: Physiological saline in equal volume to that used during the infusion of sugars, was administered orally and its effects on serum insulin and blood sugar concentrations were studied. The data are shown in Figure 13 and Table XIII. Insignificant changes ($p^* > .05$)[1], in both the serum insulin and blood sugar concentrations were noted over 2 hours of observations.

3) Glucose: Figure 14 and Table XIV illustrate the results of experiments where glucose was given orally. It should be noted that although the rise in blood glucose concentration was very small i.e. maximum rise of 86 ± 18 mgs % in comparison to the maximum rise of 527 ± 25 mgs % observed after intravenous glucose, the rise in serum insulin level i.e. 1.98 ± 0.27 fold was similar ($p < .60$) to 2.18 ± 0.20 fold increase following the intravenous infusion of glucose (Table XXII, Figure 22). Serum insulin levels increased gradually to a peak level at 30 minute time interval and declined thereafter despite the continual rise in blood glucose level until 120 minutes of administration.

4) Mannose: Previous findings of Wood et al. (63) that orally administered mannose did not appear in the blood stream are confirmed by the present findings and the results are shown in Figure 15 and Table XV. Despite the minor changes in blood mannose concentration i.e. maximum rise 25 ± 3 mgs %, serum insulin level increased by 1.72 ± 0.19 fold ($p < .01$). However, this rise in serum insulin level was less ($p < .05$) than 3.85 ± 0.87 fold increase observed

---

1. The significance of difference was determined by the analysis of variance.

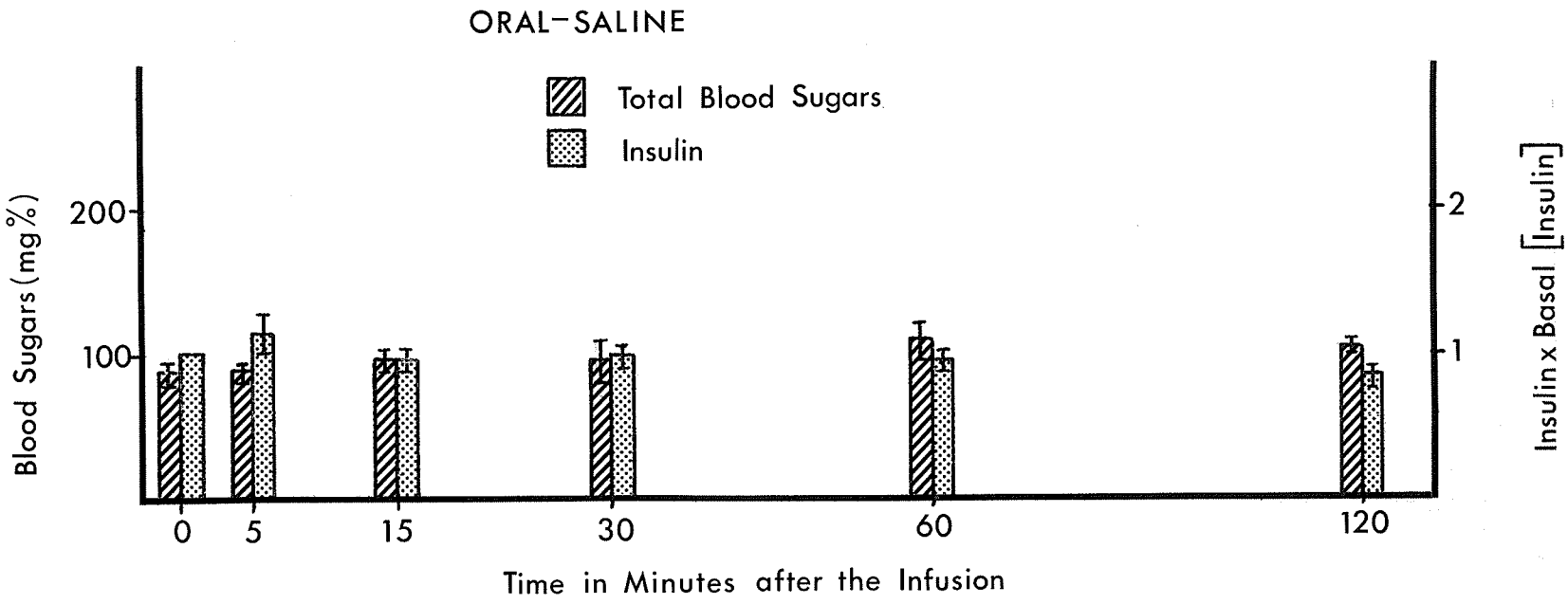

Figure 13: Effects of rapid oral administration of saline on blood sugars and serum insulin levels.

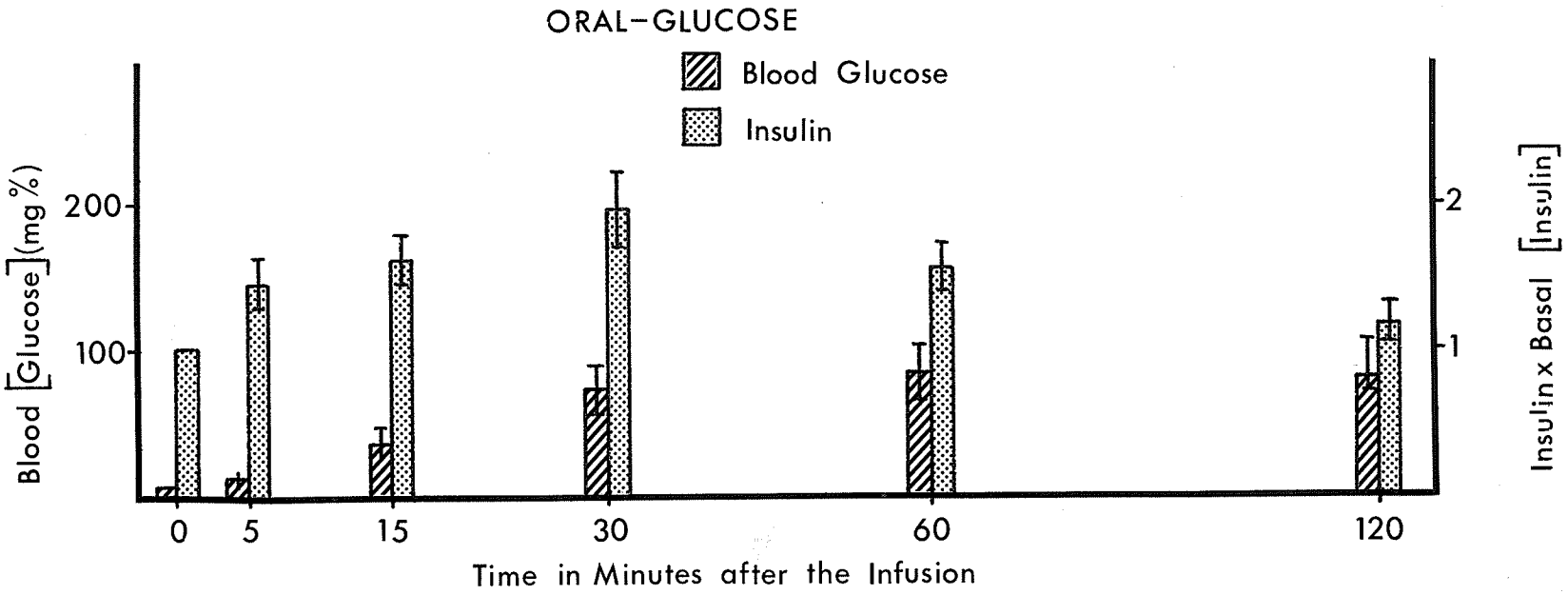

Figure 14: Effects of rapid oral administration of D(+)glucose on blood glucose and serum insulin levels.

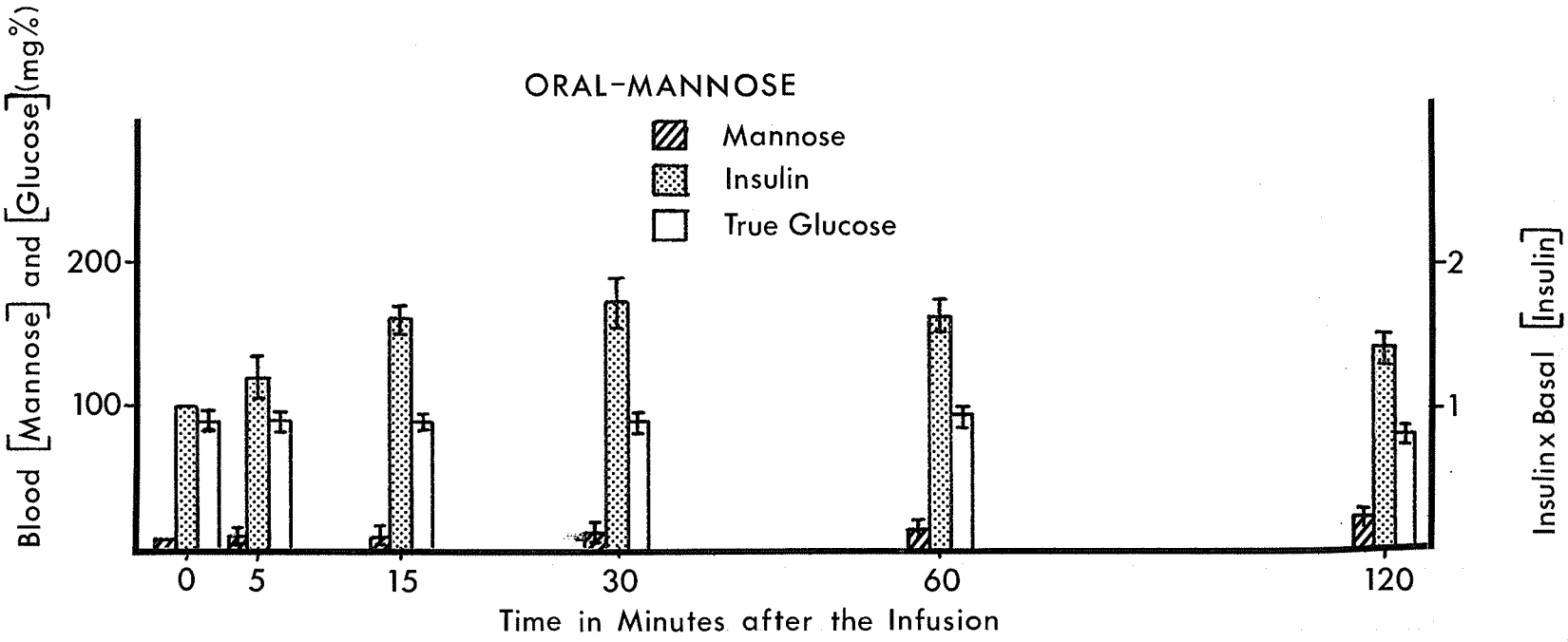

Figure 15: Effects of rapid oral administration of D(+)mannose on true blood glucose, blood mannose and serum insulin levels.

following the intravenous mannose (see Table XXII, Figure 22). Serum insulin response to oral mannose was sustained over 120 minutes of observation. The maximum rise occurred at thirty minutes, similar to that following oral glucose. Furthermore, the rise in serum insulin concentration following oral mannose was not consistent with changes in true blood glucose concentration.

5) Fructose: Figure 16 and Table XVI illustrate the findings of experiments where fructose was given orally. Despite the fact that very little of orally administered fructose appeared in the blood circulation i.e. maximum rise of 18 $\pm$ 7 mgs %, serum insulin levels increased 1.70 $\pm$ 0.08 fold ($p<.001$) compared to an increase of 2.85 $\pm$ 0.09 fold following the intravenous administration of fructose (see Table XXII, Figure 22). The insulin response to oral fructose was somewhat delayed i.e. maximum rise at 60 minutes in contrast to insulin responses to other sugars where maximum rise was usually noted at 30 minutes after the oral infusion. Some earlier investigations (65) have indicated that 58 percent of fructose given orally appear in the blood stream as glucose and 5 percent as lactic acid, and it was suggested that oral fructose was transformed into glucose during its absorption in the gut. Contrary to these findings, no significant change ($p^*>.05$) in true blood glucose level was observed. Therefore, the data suggest that the stimulation of insulin secretion was in fact due to fructose and possibly due to its presence in the gastro-intestinal tract rather than due to glucose.

6) Ribose: Figure 17 and Table XVII illustrate the effects of oral ribose on serum insulin and blood glucose concentration. It should be noted that although a very small amount (maximum rise of 69 $\pm$ 12 mgs %) of orally

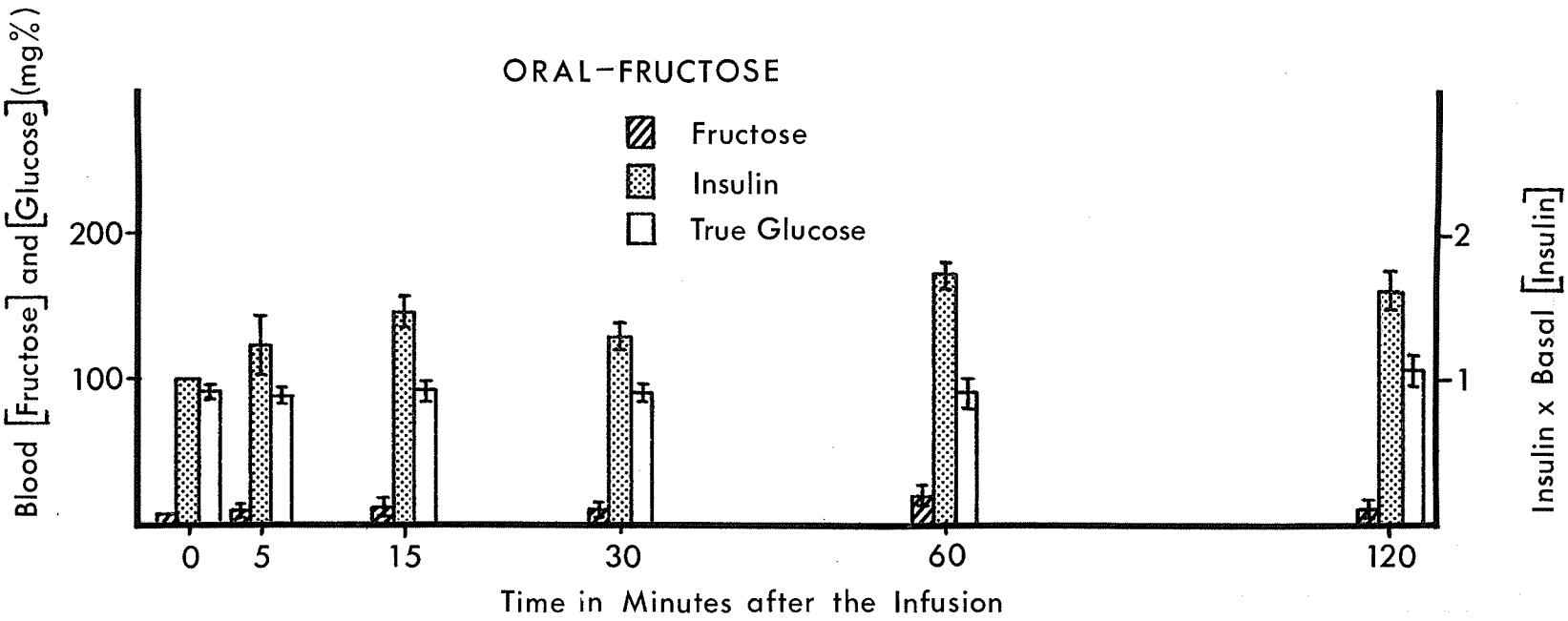

Figure 16: Effects of rapid oral administration of D-fructose on true blood glucose, blood fructose and serum insulin levels.

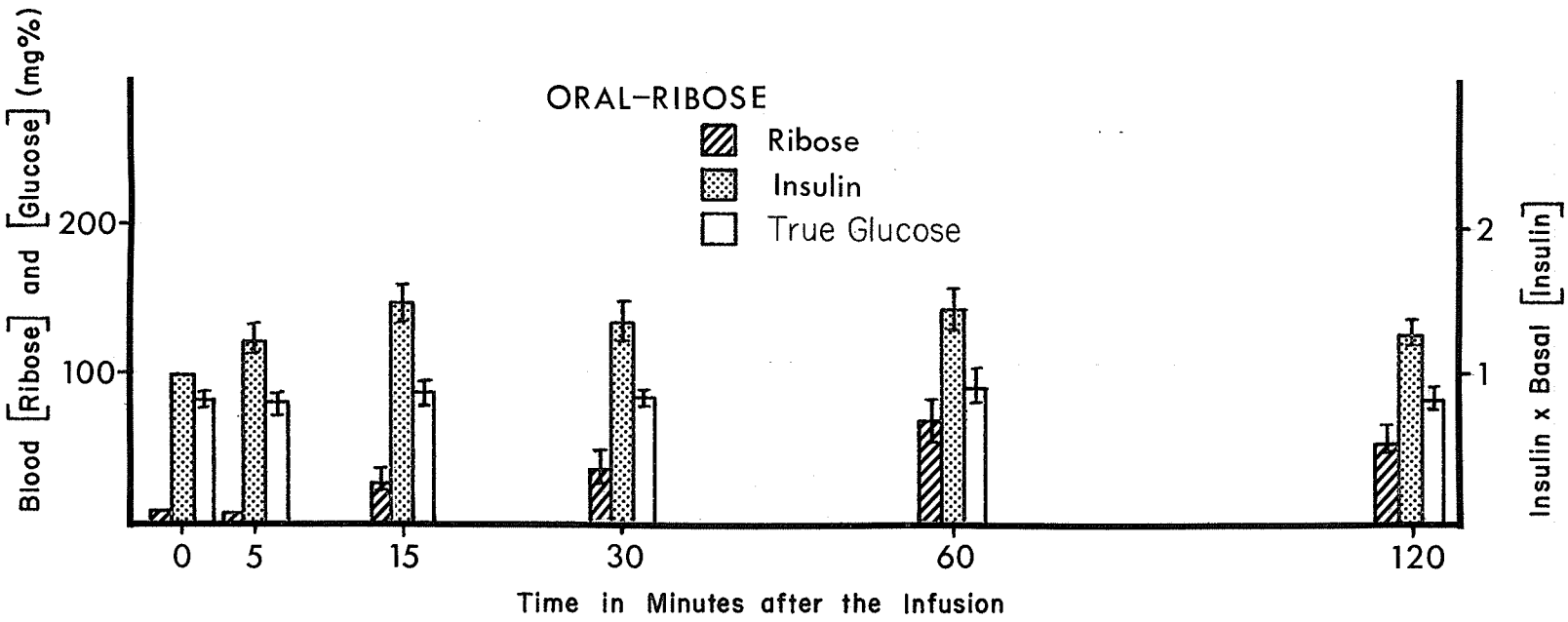

Figure 17: Effects of rapid oral administration of D(-)ribose on true blood glucose, blood ribose and serum insulin levels.

infused ribose appeared in the blood circulation in comparison to a maximum rise of 483 ± 10 mgs % following its intravenous infusion, serum insulin level increased 1.47 ± 0.11 fold. This rise was almost similar ($p < .20$) to 1.70 ± 0.08 fold increase found after the intravenous infusion of ribose (Table XXII, Figure 22). The insulin response was immediate i.e. maximum rise at 15 minutes time interval, but was sustained over 45 minutes. True blood glucose level did not change very much ($p^* > .05$) and therefore could not be the cause of this rise in serum insulin levels.

7) Galactose: The results of experiments where galactose was given orally, are shown in Figure 18 and Table XVIII. Blood galactose level increased gradually to a maximum level of 146 ± 9 mgs % at 120 minutes time interval and this rise was much smaller than a rise of 497 ± 8 mgs % observed following its intravenous infusion. However, serum insulin levels increased to a maximum level of 1.91 ± 0.24 which was not statistically different ($p < .50$) from 1.68 ± 0.14 fold rise observed after its intravenous infusion (Table XXII, Figure 22). The insulin response to oral galactose was most pronounced and gradually increased to a peak response at 60 minutes time interval and declined slightly thereafter. This insulin response was also unique in that the maximum rise in serum insulin level was somewhat greater than the maximum rise following its infusion intravenously. Oral galactose did not seem to be transformed to glucose in the small intestine because no significant change ($p^* > .05$) was observed in true blood glucose concentration during 2 hours of experiments.

8) Xylose: The results showing the effects of oral xylose on serum insulin and blood glucose levels are given in Table XIX and displayed in

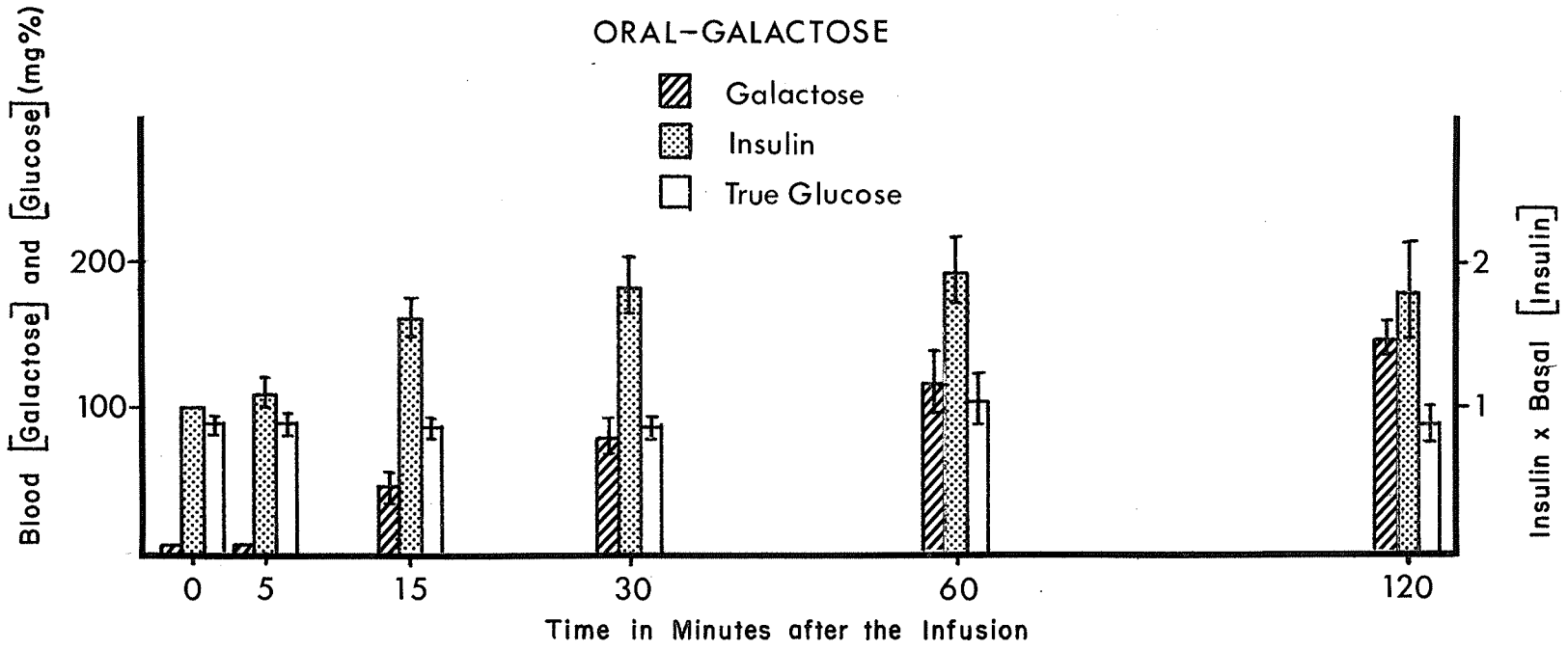

Figure 18: Effects of rapid oral administration of D(+) galactose on true blood glucose, blood galactose and serum insulin levels.

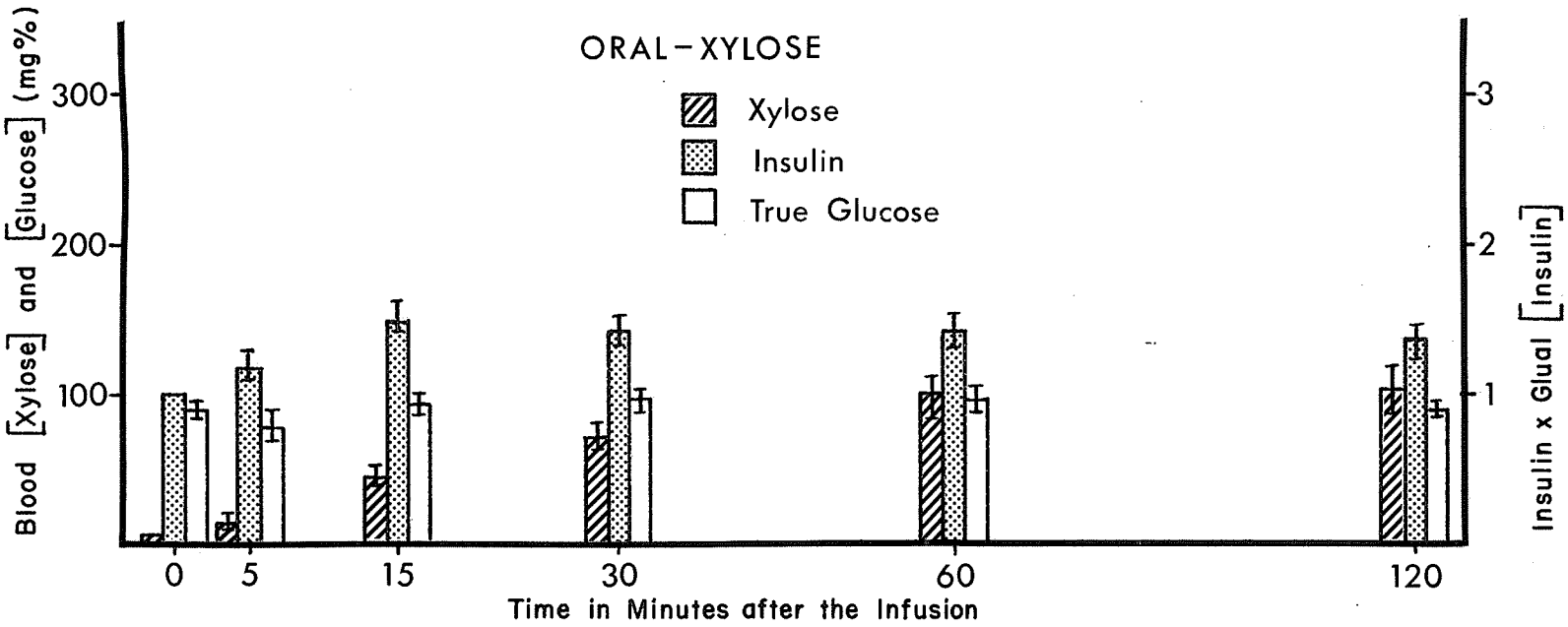

Figure 19: Effects of rapid oral administration of D-xylose on true blood glucose, blood xylose and serum insulin levels.

Figure 19. A small amount of orally administered xylose i.e. maximum rise of 103 ± 9 mgs %, appeared in the blood stream in comparison to the rise of 521 ± 15 mgs % which followed its infusion intravenously. In contrast, serum insulin level was increased by 1.53 ± 0.09 fold after oral xylose and was similar ($p<.20$) to 1.81 ± 0.21 fold increase after its administration intravenously (Table XXII, Figure 22). Insulin response to oral xylose was exactly similar to that observed following oral ribose in that it was immediate, reaching a peak response at 15 minutes and sustained for 45 minutes and a slight decrease at 120 minutes was noted. True blood glucose concentration remained constant during the course of experiments ($p^*>.05$).

9) α-methyl Glucoside: Figure 20 and Table XX show the effects of α-methyl glucoside infused orally on serum insulin and blood glucose concentrations. It is quite apparent that α-methyl glucoside given orally appeared in the blood circulation quite rapidly reaching a peak level of 320 ± 49 mgs % at 120 minutes which was highest of all the sugars tests orally (Table XXII, Figure 22). Unlike all the other sugars, α-methyl glucoside had no effect on the serum insulin levels as they were not significantly different from the mean control insulin level at any time interval i.e. p was never less than 0.2 (Table XX). Blood glucose concentrations were hardly changed ($p^*>.05$) during the two hours of observations. Both the above observations are in line with the suggestion of Wilson et al. (66) that 1) α-methyl glucoside is transported through the small intestine of hamster by an active process and 2) it does not seem to be metabolized.

10) Glycine: Since almost all the sugars except α-methyl

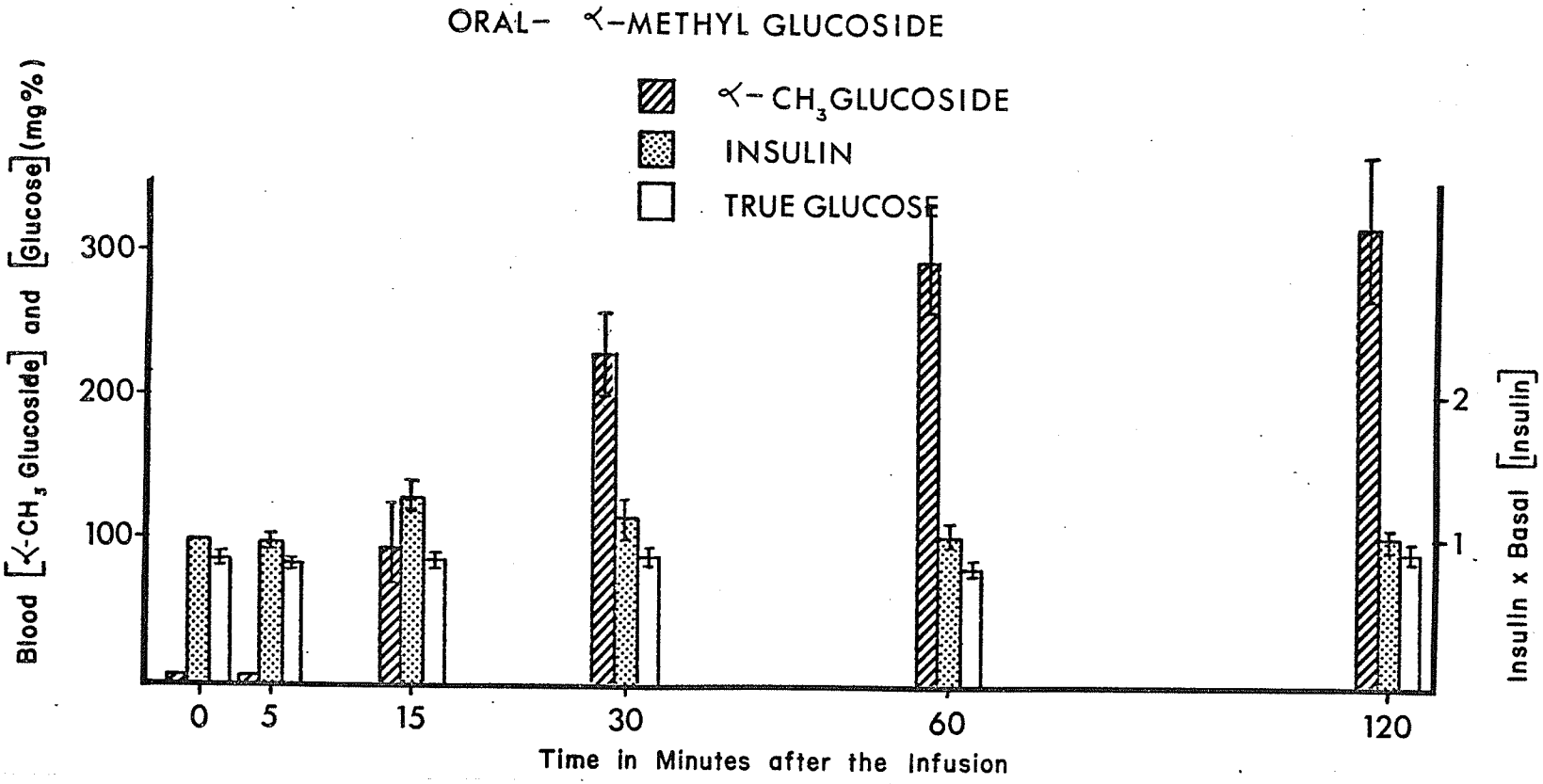

Figure 20: Effects of rapid oral administration of ∝-methyl glucoside on true blood glucose, blood ∝-methyl glucoside and serum insulin levels.

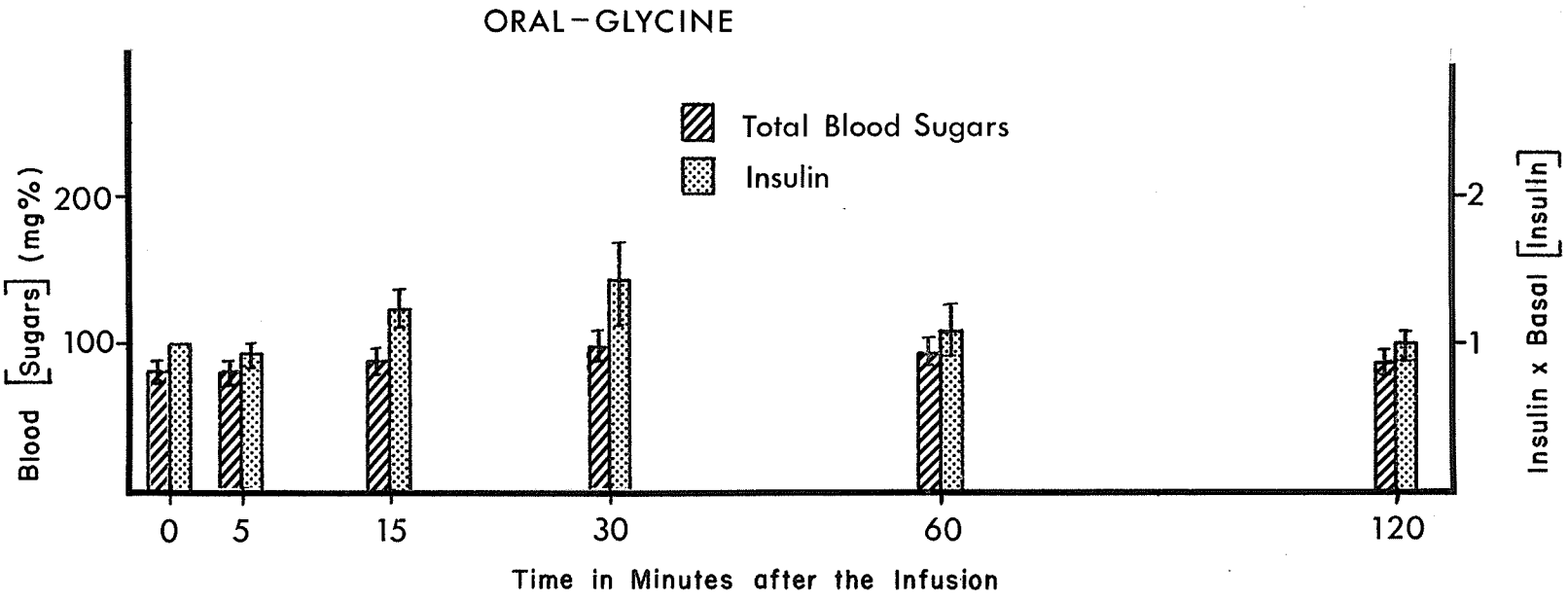

Figure 21: Effects of oral administration of glycine (Equimoles to monosaccharides) on blood sugars and serum insulin levels.

glucoside, stimulated considerable insulin secretion after their infusions orally in comparison to the insulin response following their intravenous infusion, it was of interest to establish if the mere presence of foodstuff in the gut will elicit an insulin response similar to that following oral monosaccharides. This possibility was investigated by administering glycine orally. The results of such experiments are shown in Figure 21 and Table XXI. It is quite apparent that oral glycine did not affect either the blood glucose concentration ($p^* > .05$) or the serum insulin levels i.e. p was never less than 0.20 (Table XXI.)

The data presented above clearly demonstrate that neither the process of sugar absorption nor the mere presence of foodstuff in the gut is associated with the release of insulinotropic substance from the gut. However, it demonstrates that the process of insulin release is specific to those sugars which possess an active reducing group.

SECTION V

DISCUSSION

A. EXPERIMENTAL PROCEDURES

1) Controls: During this study, 50 grams percent solutions of respective monosaccharides were used. It would appear, that the infusion of sodium chloride solution iso-osmotic with the sugar solution would have been an appropriate control. However, the preliminary experiments where equimoles of sodium chloride were administered, produced insulin and glucose response which were similar to that following the infusion of isotonic saline solution. Furthermore, similar isotonic saline control infusions have been used by a number of researchers (6-9) in investigations similar to the present study.

2) Rapid Infusion of sugars: McIntyre et al. (33) have shown that the rapid infusion of glucose intravenously and intrajejunally, produced a marked difference in insulin response. Since the purpose of the present study was to investigate these differences in oral and intravenous insulin responses, all the substances were infused rapidly. However, it would have been more desirable if glucose could have been infused intravenously at a rate such as to produce a hyperglycemia comparable to that observed after oral administration and then compare insulin responses to both routes of infusions.

B. INTRAVENOUS ADMINISTRATION OF MONOSACCHARIDES

The results of intravenous administration of monosaccharides show that a variety of sugars are capable of stimulating insulin secretion. Such an effect of monosaccharides seems to depend upon the active reducing group because α-methyl glucoside did not have stimulatory effect. However, there

were some qualitative differences among these sugars regarding their ability to bring about an elevation in serum insulin levels. Fructose and mannose had a transitory effect whereas glucose, galactose, xylose and ribose had a sustained effect.

These findings are not in complete agreement with the reports of other investigators. However, these differences could be due to the biological differences among various animal species used and different experimental procedures. This is summarized in Table XXIII.

1) Classification of Monosaccharides:

The monosaccharides studied in this investigation can be classified into different categories with respect to their potency (strong or weak stimulation), rapidity (immediate or delayed insulin response) and durability (sustained or unsustained insulin response) as follows:

Category 1: Those monosaccharides which produced marked and immediate rise in serum insulin levels after their intravenous infusion e.g. mannose and fructose. Although both fructose and mannose produced marked and immediate insulin response, they differed in that the serum insulin levels following intravenous fructose, unlike after mannose, remained significantly above the mean control insulin level. Furthermore, intravenous fructose was removed from the circulation at a rate somewhat faster than mannose.

Category 2: Those sugars which gave a moderate but prompt insulin response which extended over a longer period of time e.g. glucose and galactose. Both glucose and galactose produced a moderate and immediate

insulin response and the maximum insulin response extended from 5 to 15 minutes after the infusion. Both produced sustained stimulation of insulin secretion. However, these sugars differ in that the insulin response to glucose was somewhat greater than to galactose; furthermore, intravenous glucose was removed faster than intravenous galactose. In terms of potency and rapidity to stimulate insulin secretion, glucose can be classified in category 1. However, in terms of persistence of stimulation it fits in the seconds category quite well.

Category 3: Those sugars which produced slight insulin response immediately but the maximum response was delayed i.e. at 30 minutes after the infusion e.g. ribose and xylose. The insulin response to ribose and xylose were almost similar during the first hour after the infusion i.e. slight, immediate rise in serum insulin which gradually increased to a peak level at 30 minutes. These sugars differ in that the serum insulin level, unlike after ribose, declined immediately after the peak level following the infusion of xylose. Both sugars were removed at a similar rate and did not convert to glucose.

Category 4: Those sugars which did not stimulate insulin secretion .e.g α-methyl glucoside. This sugar analogue was removed at a rate similar to that of galactose, xylose and ribose and furthermore did not convert to glucose during 2 hours of observation.

Intravenous administration of pyruvate stimulated insulin secretion slightly. The insulin response was much delayed (peak response at 60 minutes compared to usual peak response at 5-30 minutes after the injection of other substances).

2) Specificity of Insulin Secretion:

The stimulation of insulin secretion seems to be dependent upon the active reducing group of a sugar moiety. This inference is based upon the fact that intravenous infusion of all the sugars except α-methyl glucoside, stimulated insulin secretion.

The arrangement of hydroxyl group and hydrogen atom at carbon # 2 does not seem to be related to the phenomenon of insulin secretion because galactose and mannose which differ in molecular arrangement ar carbon #2, stimulated insulin secretion. Furthermore, hydroxyl group at carbon #2 is not necessary because fructose, a ketose sugar, stimulated insulin secretion. The molecular arrangement at carbon #3 does not seem to be essential for the stimulation of insulin secretion because xylose and ribose, despite being different structurally at carbon #3, elevated serum insulin levels equally. The stimulation of insulin secretion is not related to molecular arrangement at carbon #4, because galactose and glucose produced similar insulin responses despite their structural dissimilarity at that carbon atom. The structural arrangement at carbon #5 and #6, does not seem to be responsible because pentoses as well as hexoses stimulated insulin secretion despite their structural dissimilarity at these carbon atoms.

Grodsky et al. (9) have reported that the stimulation of insulin release is not specific to molecular arrangement at carbon # 1 to # 3, since galactose, xylose and l-arabinose did not stimulate insulin secretion although they are structurally similar to glucose in this portion of the molecule. Pozza et al. (6) have also concluded that the stimulation of insulin secretion is not specific to the structure of a sugar molecule but rather to those sugars which are utilizable and insulin sensitive.

3. Discussion of Insulin Responses Individually:

A brief discussion of individual insulin responses to different sugars seems essential and is given in this section.

i) Glucose: It has been shown that intravenous infusion of glucose enhances the release of insulin (6, 7, 8, 11). Furthermore, it is known that intravenous glucose stimulates insulin secretion greater than other substances (6, 7, 11). However, the evidence regarding its latter property is conflicting since Karam et al. (8) have recently reported that intravenous mannose raised serum insulin levels greater than glucose in man. Likewise, it was observed in the present study that intravenous mannose raised serum insulin levels greater than glucose in rabbits. However, the insulin response to glucose, unlike mannose, was sustained over two hours of observation. Kuzuya et al. (16) have also shown that intravenous xylitol stimulated insulin secretion greater than glucose in dogs. These varieties of insulin responses to intravenous glucose can be explained in terms of biological differences among different animals and different experimental procedures used.

ii) Mannose: The present findings that mannose stimulates insulin secretion are in line with those of Karam et al. (8), in vitro studies of Coore et al. (12), Malaisse et al. (13) and the perfusion studies of Grodsky et al. (9) and Sussman et al. (10). However, these are in sharp contrast to those of Pozza et al. (6) and Sheps et al. (61). The indices of insulin secretion used in the latter studies differed from all other studies and probably explain the different findings. The fact that insulin response to intravenous mannose was very transient can be explained by the findings that it was removed very rapidly from the blood circulation

and had limited time to act on the β-cell. These findings support the hypothesis of Harding et al. (63) that there is little or no renal threshold for mannose and most of it is excreted in the urine. True blood glucose level did not change significantly ($p^* > .05$) after the intravenous infusion of mannose. This suggests that mannose stimulated insulin secretion either by its direct effect on the B-cell or by some mechanism other than its transformation into glucose. The other possible stimuli are: 1) a metabolite of mannose 2) a hepatic factor which has been shown (11) to respond to the changes in hepato-portal blood sugar concentration.

iii) Fructose: Intravenous fructose stimulates insulin secretion immediately and markedly. These findings are consistent with those of Goetz et al. (11), and perfusion studies of Grodsky et al. (9) and Sussman et al. (10). However, these were in disagreement with those of Pozza et al. (6), in vitro studies of Malaisse et al. (13) and Coore et al. (12). Contrary to the findings of Boda (7), no rise in true blood glucose level was found during 2 hours of observation in this study, and therefore suggests that intravenous fructose stimulated insulin secretion by some mechanism other than its conversion to glucose. The other stimuli can be: 1) a metabolit of fructose 2) a hepatic factor which responds to the changes in sugar concentration. 3) its direct effect on the pancreas.

iv) Galactose: Conflicting data has been reported regarding the stimulatory effect of galactose on the β-cells of pancreas. The present findings that intravenous galactose elevated serum insulin levels in rabbits agree with those of Boda (7), Goetz et al. (11) and of Pozza et al. (6) but were in sharp contrast to the data reported by Karam et al. (8) and in vitro data of Grodsky et al. (9), Sussman et al. (10), Coore et al. (12) and of Malaisse et al. (13).

True blood glucose levels were stable following the intravenous infusion of galactose thus confirming the previous findings of Boda (7). Furthermore, the changes in serum insulin levels did not correspond closely to the changes in blood galactose concentrations. Therefore the data suggest that 1) the conversion of galactose to glucose was not responsible for the stimulation of insulin secretion. 2) some other stimuli besides the rise in blood galactose level were present. It is plausible to suggest that stimuli such as a metabolite of galactose or its metabolism in the liver are equally possible. The maximum insulin response to intravenous galactose was somewhat delayed in contrast to immediate maximum insulin response to glucose, fructose and mannose. This difference is probably due to the poor metabolism of galactose in comparison to other sugars. The sustained stimulation of insulin secretion by intravenous galactose is consistent with the findings that it was removed slowly. Slow removal of galactose may predict its poor metabolism or its minimum disposal through urine.

v) Ribose: Intravenous infusion of ribose brought forth an immediate rise in serum insulin level which continued until a peak level at 30 minutes. Serum insulin levels remained elevated thereafter. These findings were in accord with those of Goetz et al. (11) and of Pozza et al. (6) but disagree with in vitro data of Coore et al. (12) and Malaisse et al. (13). The finding that true blood glucose level did not change significantly after the intravenous infusion of ribose, suggests that ribose did not stimulate insulin secretion by transforming glucose. Despite the presence of ribose in small amounts in the blood, sustained stimulation of insulin secretion supports the hypothesis that insulin secretion is related to the metabolism of the sugar infused. Contrary to

the findings of Segal et al. (62), hypoglycemia did not follow the intravenous infusion of ribose in rabbit. This is probably either due to rapid conversion of ribose to glucose to compensate any decrease in true blood glucose level or due to different responses by different animals.

vi) Xylose: A number of investigators (6, 9, 12, 13) have shown that xylose did not stimulate the release of insulin. Contrary to these findings, infusion of xylose intravenously, elevated serum insulin levels in the present study. The insulin response was delayed and somewhat sustained despite the small amounts of xylose in the blood. Since xylose is poorly metabolized (75, 76), this delayed and sustained insulin response probably indicates that elevation of serum insulin levels was somehow associated with the metabolism of xylose. True blood glucose level did not change significantly indicating that the stimulatory effect of xylose was not due to its conversion to glucose.

vii) α-methyl Glucoside: Intravenous infusion of α-methyl glucoside did not elevate serum insulin levels significantly. Since this was the only sugar which did not stimulate insulin secretion and lack an active reducing group, it was concluded that an active reducing group of a sugar moiety is essential for the stimulation of insulin secretion.

viii) Pyruvate: Intravenous administration of pyruvate elevated serum insulin levels slightly. The serum insulin level increased gradually to a peak level at 60 minutes after the injection. It is logical to assume that the blood pyruvate level at the time of peak insulin response was lower than at 5-15 minutes after the injection. This latter assumption leads one to suggest that pyruvate had no direct stimulatory effect on the pancreas, and probably its

metabolism through different metabolic pathways was responsible for the elevation of the serum insulin levels. Minor changes in true blood glucose levels were noted. However, these were considered insignificant ($p^* > .05$) to be responsible for the stimulation of insulin secretion.

4. Conclusions:

a) All sugars when infused intravenously i.e. mannose, fructose, glucose, xylose, ribose and galactose with the exception of α-methyl glucoside, stimulated insulin secretion. Pyruvate stimulated insulin secretion slightly.

b) Elevation of serum insulin level required an active reducing group of a sugar molecule but was non specific to the molecular arrangement at carbon #2 to #6 of a sugar moiety.

c) Stimulation of insulin secretion seems to be related to the rise in blood sugar concentration as well as to the rate of metabolism of the sugar infused.

C. ORAL ADMINISTRATION OF MONOSACCHARIDES

1. Discussion of Insulin Responses Individually

i) Glucose: In the present study it was observed that although the rise in blood glucose level after its oral administration was not nearly as much as after intravenous administration, serum insulin levels increased by the same extent after both routes of administration. These results are similar to those of McIntyre et al. (32, 33) and of Elrick et al. (34). However, these data differed from that of McIntyre et al. (33) in that they noted a greater rise in serum insulin levels after oral glucose than following the intravenous infusion of glucose. To explain their findings, they postulated that a humoral substance is released from the small

intestine during the absorption of intrajejunal glucose which stimulates insulin secretion in addition to hyperglycemia. Further studies of Lawrence et al. (48) and Samols et a. (43) have shown that circulating levels of glucagon were increased following the oral infusion of glucose whereas no such change in glucagon levels was observed after intravenous administration of glucose. It was suggested that the rise in glucagon level was probably responsible for a greater insulin response to oral glucose. The discrepancy between the present data and that reported by McIntyre et al. (33) may be due to species differences. The present findings can be explained on the assumption that an additional stimulus is associated with oral glucose and produce a better insulin response to oral glucose then to intravenous glucose. The site from which this additional stimulus is elicited is not certain. However, it has been suggested to be either in the small intestine (33) or the liver (11). In the present study, the stomach could also be a possible site of release of this humoral substance.

ii) Fructose: Despite negligible increase in blood fructose concentration after its oral administration, serum insulin levels were increased considerably. If the rise in blood fructose concentration were the sole stimulus to the pancreas, the rise in serum insulin level cannot be accounted for by this small increase in blood fructose concentration. True blood glucose levels remained unchanged and therefore, cannot be considered responsible for this rise in serum insulin level. The presence of an additional stimulus is obvious and it seems that it responds to oral fructose also. The small rise in blood fructose level following its oral administration can be accounted for by the fact that fructose is absorbed at a much slower rate than glucose (67) and metabolized faster than glucose (68, 69).

iii) Mannose: There was hardly any rise in blood mannose concentration following its oral administration. However, serum insulin level increased considerably. This small rise in blood mannose level following its administration orally, can be explained by the fact that mannose is transported across the wall of small intestine at a slower rate than glucose (63, 67) and also by the fact that it is removed faster than glucose from the blood stream (63). Furthermore, this is in line with the findings that mannose is transported by a passive process (66). In order to explain greater rise in serum insulin level relative to the rise in blood mannose level after its oral administration, one must postulate the presence of additional stimulus to the pancreas which brings about a greater release of insulin. True blood glucose level did not change very much and cannot be considered responsible for this insulin response. The findings that there was no rise in true blood glucose level, are in accord with those of Drury et al. (70).

iv) Galactose: The insulin response to oral galactose differed from the other sugars in that the maximum rise in serum insulin level was greater than the maximum rise after intravenous infusion of galactose despite a smaller rise in blood galactose concentration in comparison with that observed following intravenous galactose. In order to account for such an insulin response to oral galactose with little rise in blood galactose level, one must postulate an additional stimulus to the pancreas. Galactose given orally did not seem to convert to glucose during its absorption across the intestinal wall because there were insignificant changes in true blood glucose concentration. However, its conversion to some other metabolites (galactose 6-phosphate or lactate) cannot be ruled out from the data of this study. Its appearance in the blood stream following its oral

administration seems to support the findings of Wilson et al. (66) that galactose is transported by an active process and by similar mechanism as glucose (77). Since its rate of removal was slower (present findings) but the rate of absorption faster than glucose (67), its greater rise in the blood stream than glucose becomes self explanatory. Sustained insulin response to oral galactose is due to its absorption over a longer period of time.

v) Xylose: The rise in blood xylose concentration following its administration orally was much less than the rise following intravenous xylose. However, the rise in serum insulin level after oral xylose was similar to that observed following intravenous xylose (Table XXII, Figure 22). Contrary to the findings of Wyngaarden et al. (71), true blood glucose level did not change significantly and therefore does not seem to be responsible for the rise in serum insulin level after oral xylose. Blood xylose level gradually increased indicating its transport against the concentration gradient as has been shown by Czaky et al. (72).

vi) Ribose: Despite a small rise in blood ribose concentration after its oral administration when compared with that observed following intravenous infusion, serum insulin levels were elevated to the same extent after both routes of administration. True blood glucose levels did not change significantly and these findings are in sharp contrast with those of Beaconsfield et al. (73) who found hypoglycemia following the infusion of ribose orally. It has been shown that ribose is transported by diffusion process (66). However, in the present study, blood ribose concentration increased gradually to a peak level at 60 minutes and declined slightly thereafter. A similar maximum rise at 60 minutes after the oral infusion

was reported by Beaconsfield et al. (73). This suggests that ribose was absorbed against a concentration gradient and probably at a rate much slower than glucose. Sustained stimulation of insulin secretion followed oral ribose which was not correlated with the amount of blood ribose present in the circulation. This out of proportion insulin response can only be explained if one postulates the presence of an additional stimulus which responds to the oral administration of ribose.

vii) α-methyl Glucoside: A marked rise in blood α-methyl glucoside followed its administration orally in comparison to the increase observed following the administration of other sugars. However, serum insulin levels, unlike other sugars, did not change significantly. The study of this sugar was interesting in that it explained the differences in rises of orally administred sugars. Since α-methyl glucoside is shown to be actively transported and a non-metabolizable sugar (66), it appeared in largest amounts in the blood circulation of all the other sugars administered orally. True blood glucose levels remained constant indicating that α-methyl glucoside was not transformed to glucose during its intestinal absorption. These findings suggest that the appearance of orally administered sugars in the blood circulation depends upon 1) their rate and mechanism of intestinal absorption and 2) their rate of removal. These data further suggest that the release of insulinotropic substance is not associated with either the rate or the mechanism of intestinal absorption but seems specific to the active reducing group of a sugar molecule.

viii) Glycine: Oral administration of glycine did not change either the blood sugar levels or serum insulin levels during 2 hours of observations. These findings indicate that the mere presence of nutrient in the gastro-intestinal tract

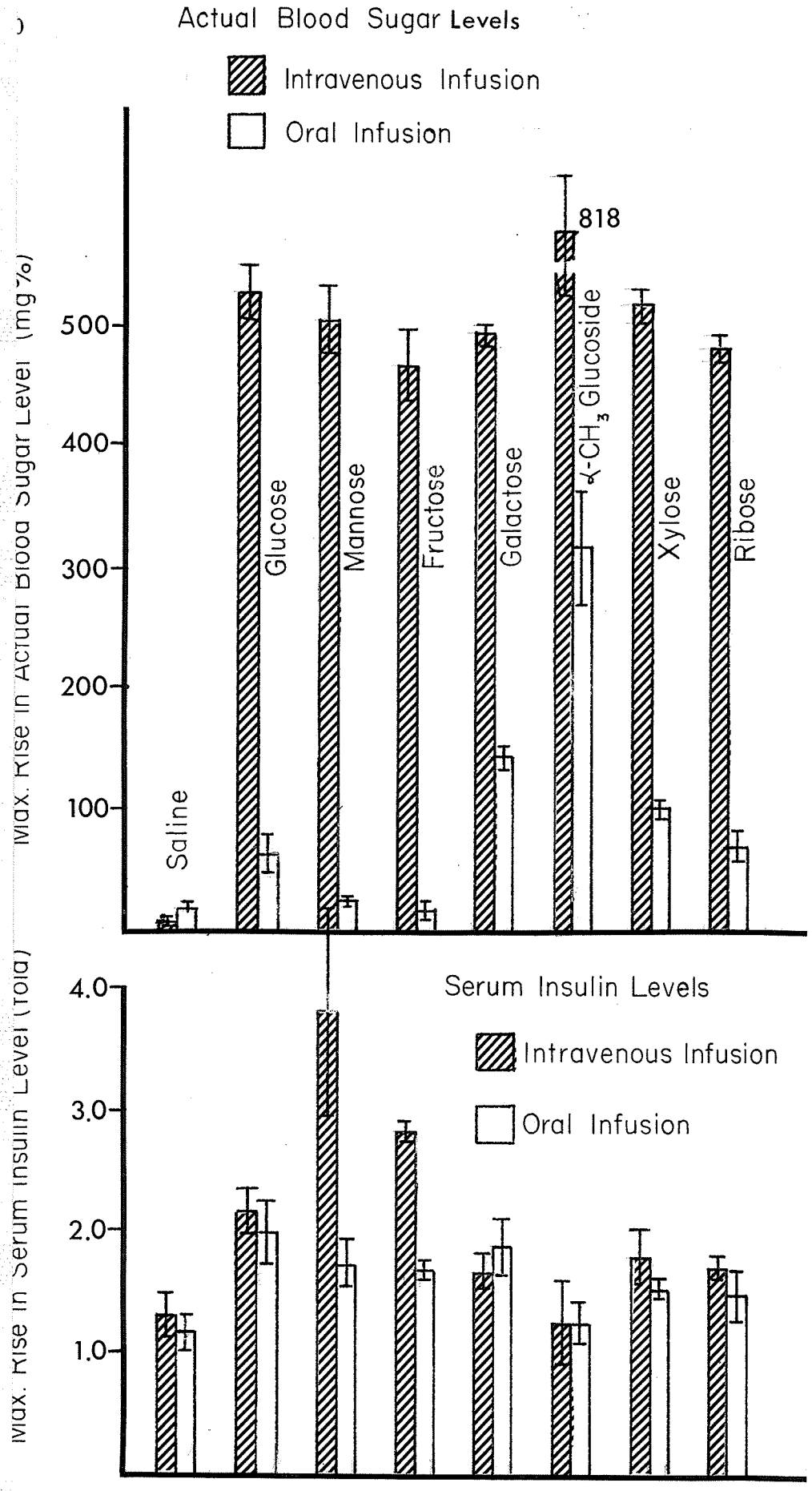

Figure 22: Depicts the comparison of maximum increases in serum insulin levels (intravenous versus oral) and in actual blood sugar concentration (intravenous versus oral).

did not cause the release of this insulinotropic substance but that the release is quite specific to monosaccharides possessing an active reducing group.

2) Comparison of Oral and Intravenous Insulin Responses

If one compares oral with intravenous insulin response to each sugar, one can classify these monosaccharides into categories similar to those of intravenous insulin responses. However, the sequence is little changed, in that glucose and galactose gave higher insulin response than fructose and mannose (see Table XXII, Figure 22 for comparison).

Category 1: Those sugars which produced insulin response to their oral infusions which was similar to intravenous insulin response e.g. Glucose and Galactose. It is apparent from the data that oral glucose and galactose stimulated insulin secretion markedly and equally. Both sugars produced sustained insulin responses and both seem to be transported by the same mechanism thereby supporting the earlier findings of Fisher et al. (74). However, they differed in that the blood galactose level increased to a maximum level which was greater than glucose. These observations are in line with the findings of Cori et al. (67) that galactose is transported at a faster rate than glucose in rat and that galactose is removed at a slower rate than glucose in rabbits (present findings). The maximum rise in serum insulin level occurred at 30 and 60 minutes after the infusion of glucose and galactose respectively.

Category 2: Those sugars which produced insulin response significantly lower than that found after intravenous infusion e.g. Mannose and Fructose. Both sugars stimulated insulin secretion considerably and equally. However, the maximum rise in serum insulin level following oral administration was significantly

lower than that seen after intravenous infusion. This was probably associated with the lack of rise in blood fructose and mannose concentrations after their oral infusion.

Category 3: Oral administration of sugars which stimulated the pancreas moderately but the insulin response was similar to that after intravenous administration e.g. Ribose and Xylose. The insulin response to pentoses occurred sooner than to hexoses. Sustained stimulation of insulin secretion predicts the limitation of intestinal absorption for the entry of sugar into the blood circulation.

Category 4: The sugar which did not stimulate insulin secretion when administered orally e.g. α-methyl glucoside. Oral infusion of α-methyl glucoside did not stimulate insulin secretion and the insulin responses to both routes of administration were similar.

Glycine when injected orally did not stimulate insulin secretion although slight but insignificant rise in serum insulin level was observed.

3. Specificity:

It is apparent from the data that the increments in the levels of all blood sugars after their oral administration, were smaller than the corresponding levels after their intravenous infusions. However, serum insulin levels were elevated to a similar degree after both routes of sugar administration, with the exception of mannose and fructose when serum insulin levels after oral administration were somewhat lower than those after the intravenous infusion. To explain such findings, an additional stimulus is proposed which responds to sugars administered orally. Possible sites of its elicitation are stomach, small intestine and the liver. The different views regarding the site of this additional mechanism i.e. intestinal or

hepatic factor, can be reconciled by the recent proposition of Anderson et al. (79) who have shown by sampling the hepatic-vanous and pancreatic-venous blood every 15 seconds both in dog and man, that the fluctuations in hepatic-venous blood glucose concentration are followed by parallel changes in the pancreatic-venous insulin levels. Furthermore, a parallel rise in glucagon-like-activity in the pancreatic-venous blood was seen to precede the rises in hepatic glucose output. Therefore, it was proposed from these findings that probably glucagon is released either from the pancreas or from the small bowel wall during the post absorption of nutrient which then enhances the output of glucose from the liver and the glucose in turn stimulates the release of insulin from the pancreas. The present findings are not conclusive about the site of this additional mechanism and therefore, it can be either one or all of the three possibilities cited above.

It is apparent from these findings that this additional stimulus responds to only those sugars which possess an active reducing group. The molecular arrangement at carbon #2 to #6 does not seem to be a prerequisite for a sugar to stimulate this additional mechanism. Furthermore, it is not provoked by the mere presence of nutrient in the gut and does not seem to be related to the rate and the mechanism of intestinal transport.

4. Conclusions:

a) Insulin response to oral sugars was similar to that observed after their intravenous infusion despite the lower blood levels of these monosaccharides after their oral administration. To explain these findings, the release of an insulinotropic substance either from the stomach, small intestine or liver is proposed.

b) The release of this insulinotropic substance seems specific to those sugars which have an active reducing group. Neither the mere presence of food stuff in the gastro-intestinal tract nor the rate or mechanism of intestinal absorption appears to be responsible for the elicitation of this additional mechanism.

c) The molecular arrangement at carbon #2 to #6 of glucose molecule does not seem to be of importance in determining the capacity of an oral sugar to stimulate insulin secretion.

SECTION VI

BIBLIOGRAPHY

1. Soskin, S.; and Levine, R.: Carbohydrate Metabolism, (University of Chicago Press. 1st Edition, 1951), p.p. 248-263.
2. Levine, R.: Production, secretion and availability of Insulin Ann. Rev. Med. 15:413, 1964.
3. Williams, R.H.; and Ensinck, J.W.: Secretion, fates and actions of Insulin and related products. Diabetes 15:623, 1966.
4. Banting, F.G. and Best, C.H.: Internal secretion of pancreas. J. Lab. & Clin. Med. 7:251, 1922. and Pancreatic extracts. J. Lab. & Clin. Med. 7:464, 1922.
5. Lacy, P.E.: Pancreatic Beta Cell. Ciba Found. Colloq. Endocr. 15:79, 1964.
6. Pozza, G.; Galansino, G.; Hoffeld, H.; and Foa, P.P.: Stimulation of Insulin output by Monosaccharides and Monosaccharides derivatives. Amer. J. Physiol. 192:497, 1958.
7. Boda, J.M.: Effect of fast and hexose injection on serum Insulin concentration of sheep. Amer. J. Physiol. 206:419, 1964.
8. Karam, J.H.; Sebastiano, G.G.; Wegienka, L.C.; Grodsky, G.M.; and Forsham, P.H.: Effect of selected hexoses, of epinephrine and of glucagon on Insulin secretion in man. Diabetes 15:571, 1966.
9. Grodsky, G.M.; Batts, A.A.; Bennett, L.L.; Vcella, C.; McWilliams, N.B.; and Smith, D.F.: Effects of carbohydrates on secretion of Insulin from isolated rat pancreas. Amer. J. Physiol. 205:638, 1963.

10. Sussman, K. E.; Vaughan, G.D., and Timmer, R. F.: An in vitro method for studying insulin secretion in the perfused isolated rat pancreas. Metabolism 15:466, 1966.

11. Goetz, F.C.; Maney, J.W., and Greenberg, B. Z.: The regulation of Insulin secretion: Effects of the infusion of glucose, ribose and other sugars into the portal vein of dogs. J. Lab. & Clin. Med. 69:537, 1967.

12. Coore, H.G., and Randle, P.J.: Regulation of Insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. 93:66, 1964.

13. Malaisse, W.; Malaisse-Lagae, F., and Wright, P.H.: A new method for the measurement in vitro of pancreatic insulin secretion. Endocr. 80:99, 1967.

14. Kosaka, K.; Ide, T,; Kuzuya, T.; Miki, E.; Kuzuya, N., and Okinaka, S.: Insulin-like-activity in pancreatic vein blood after glucose loading and epinephrine hyperglycemia. Endocr. 75:9, 1964.

15. R-Candela, J.L., and Garcia-Fernandez, M.C.: Stimulation of secretion of Insulin by adenosine triphosphate. Nature 197:210 and 1304, 1963.

16. Kuzuya, T.; Kanazawa, Y., and Kosaka, K.: Plasma Insulin response to intravenously administered xylitol in dogs. Metabolism 15:1149, 1966.

17. Brahmachuri, H.D., and Sarma, G.R.: Effects of injections of some intermediary metabolites on the insulin response of normal rabbits. Nature 191:491, 1961.

18. Mebane, D., and Madison, L.L.: The hypoglycemic effect of ketone bodies. J. Clin. Invest. 41:1383, 1962.

19. R-Candela, J.L.; R-Candela, R.; Hernandez, D.M., and Cortezar, T.C.: -hydroxy butyrate and sodium citrate as stimuli of the in vitro secretion of Insulin. Nature 195:711, 1962.

20. Floyd, J.C. Jr.; Fajans, S.S.; Conn, J.W.; Knopf, R.F., and Rull, J.: Stimulation of Insulin secretion by amino acids. J. Clin. Invest. 45:1487, 1966.

21. Rabinowitz, D.; Merimee, T.J.; Burgess, J.A., and Riggs, L.: Growth hormone and insulin release after arginine: Indifference to hyperglycemia and epinephrine. J. Clin. Endocr. & Metab. 26:1170, 1966.

22. Berger, S. and Vongaraya, N.: Insulin response to ingested protein in Diabetes. Diabetes 15:303, 1966.

23. Fajans, S.S.; Floyd, J.C. Jr.; Knopf, R.F., and Conn, J.W.: A comparison of leucine - and acetoacetate-induced hypoglycemia in man. J. Clin. Invest. 43:2003, 1964.

24. Floyd, J.C. Jr.; Fajans, S.S.: Knopf, R.F., and Conn, J.W.: Evidence that insulin release is the mechanism for experimentally induced leucine hypoglycemia in man. J. Clin. Invest. 42:1714, 1963.

25. Floyd, J.C. Jr.; Fajans, S.S.; Rull, J.; Knopf, R.F.; Kirsh, M.M., and Conn, J.W.: Direct Evidence that Leucine induces release of pancreatic insulin. Diabetes 14 # 7:439, 1965.

26. Scow, R.O. and Cornfield, J.: Quantitative relations between the oral and intravenous glucose tolerance curves. Amer. J. Physiol. 179:435, 1954.

27. Arn ould, Y.; Bellens, R.; Frankson, J.R.M., and Conrad, V.: Insulin response and glucose-C^{14} disappearance rate during the glucose tolerance test in the unanesthetized dog. Metabolism 12:1122, 1963.

28. Dupre, J.: Effect of route of administration on disposal of glucose loads. J. Physiol. 175:58P, 1964.

29. Dupre, J.: An intestinal hormone affecting glucose disposal in man. Lancet ii: 672, 1964, September 26.

30. Dupre, J., and Beck, J.C.: Effect of an intestinal mucosal extract on glucose disposal and serum insulin-like-activity in man. Diabetes 14:440, 1965.

31. Dupre, J. and Beck, J.C.: Stimulation of release of insulin by an extract of intestinal mucosa. Diabetes 15:555, 1966.

32. McIntyre, N.; Holdsworth, C.D. and Turner, D.S.: New interpretation of oral glucose tolerance. Lancet ii: 20, 1964, July 4.

33. McIntyre, N.; Holdsworth, C.D. and Turner, D.S.: Intestinal factors in the control of insulin secretion. J. Clin. Endocr. 25:1317, 1965.

34. Elrick, H.; Stimmler, L.; Hlad, C.J.; and Arai, Y.: Plasma insulin response to oral and intravenous glucose administration. J. Clin. Endocr. 24:1076, 1964.

35. Pfieffer, E.F.; Telib, M.; Ammon, J.; Melani, F., and Ditschuneit, H.: Letter to the Editor, Diabetologia 1:131, 1965.

36. Unger, R.H.; Ketterer, H.; Eisentraut, A., and Dupre, J.: Effect of secretin on insulin secretion. Lancet ii: 24, 1966, July 2.

37. Unger, R.H.; Ketterer, H.; Dupre, J., and Eisentraut, A.M.: The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. J. Clin. Invest. 46:630, 1967.

38. Dupre, J.; Rojas, L.; White, J.J.; Unger, R.H., and Beck, J.C.: Effect of secretin on insulin and glucagon in portal and peripheral blood in man. Lancet ii:26, 1966, July 2.

39. Boyns, D.R.; Jarrett, R.J., and Keen, H.: Intestinal hormones and plasma insulin. Lancet i:409, 1966, February, 19.

40. Meade, R.C.; Kneubuhler, H.A.; Schulte, W.J., and Barboriak, J.J: Stimulation of insulin secretion by pancreozymin. Diabetes 16:141, 1967.

41. Unger, R.H.,; Ketterer, H., and Eisentraut, A.M.: Distribution of immunoassayable glucagon in gastrointestinal tissues. Metab. Clin. & Expertal. 15 # 10:865, 1966, October.

42. Samols, E.; Marri, G., and Marks, V.: Promotion of insulin secretion by glucagon. Lancet ii:415, 1965, August 28.

43. Samols, E.; Tyler, J.; Marri, G., and Marks, V.: Stimulation of glucagon secretion by oral glucose. Lancet ii:1257, 1965, December 18.

44. Crockford, P.M.; Porte, D. Jr.; Wood, F.C. Jr., and Williams, R.H.: Effect of glucagon on serum insulin, plasma glucose and free fatty acids in man. Metabolism 15:114, 1966.

45. Campbell, J., and Rastogi, K.S.: Effects of glucagon and epinephrine on serum insulin and insulin secretion in dogs. Endocr. 79:830, 1966.

46. Turner, D.S., and McIntyre, N.: Stimulation by glucagon of insulin release from rabbit pancreas in vitro. Lancet i: 351, 1966, February 12.

47. Vecchio, D.; Luyckx, A.; Zahnd, G.R., and Renold, A.E.: Insulin release induced by glucagon in organ cultures of fetal rat pancreas. Metabolism 15:577, 1966.

48. Lawrence, A.M.: Radioimmunoassayable glucagon levels in man: Effects of starvation, hypoglycemia, and glucose administration. Proc. Nation. Acad. Sci: 55:316, 1966.

49. Porte, D. Jr.; Graber, A.L.; Kuzuya, T. and Williams, R.H.: The effect of epinephrine on immunoreactive insulin levels in man. J. Clin. Invest. 45:228, 1966.

50. Porte, D. Jr.: A receptor mechanism for the inhibition of insulin release by epinephrine in man. J. Clin. Invest. 46:86, 1967.

51. Porte, D. Jr.: Beta Adrenergic stimulation of insulin release in man. Diabetes 16:150, 1967.

52. Daniel, P.M., and Henderson, J.R.: The effect of vagal stimulation on plasma insulin and glucose levels in the baboon. J. Physiol. 192:317, 1967.

53. Kaneto, A.; Kosaka, K., and Nakao, K.: Effect of stimulation of the vagus nerve on insulin secretion. Endocrinology 80:530, 1967.

54. Milner, R.D.G., and Hales, C.N.: The role of calcium and magnesium in insulin secretion from rabbit pancreas studied in vitro. Diabetologia 3:47, 1967.

55. Grodsky, G.M., and Benett, L.L.: Effect of glucose "pulse", glucagon and the cations Ca^{++}, Mg^{++}, and K^{+} on insulin secretion in vitro. J. Clin. Invest. 45:1018, 1966.

56. Madison, L.L.; Mebane, D.; Unger, R.H., and Lochner, A.: The hypoglycemic action of ketones. II: Evidence for a stimulatory feedback of ketones on the pancreatic beta cell.s J. Clin. Invest. 43:408, 1964.

57. Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375, 1944.

58. Huggett, A. St. G., and Nixon, D.A.: Enzymatic determination of blood glucose. Biochem. J. 66:12P, 1957.

59. Soeldner, J.S., and Slone, D.: Critical variables in the radioimmunoassay of serum insulin using the double antibody technique. Diabetes 14:771, 1965.

60. Meade, R.C., and Klitgaard, H.M.: A simplified method for immunoassay of human serum insulin. J. Nuclear Med. 3:407, 1962.

61. Sheps, M.C.; Nickerson, R.J.; Dagenais, Y.M.; Steinke, J.; Martin, B.M., and Renold, A.E.: Measurement of small quantities of insulin-like-activity using rat adipose tissue.II: Evaluation of performance. J. Clin. Invest. 39#9:1499, 1960.

62. Segal, S.; Foley, J., and Wyngaarden, J.B.: Hypoglycemic effect of D-ribose in man. Proc. Soc. Expt. Biol. & Med. 95:551, 1957.

63. Harding, V.J.; Nicholson, T.F., and Armstrong, A.R.: Cutaneous blood sugar curves after the administration of fructose, mannose and xylose. Biochem. J. 27:2035, 1933.

64. Wood, F.C. Jr., and Cahill, G.F. Jr.: Mannose Utilization in man. J. Clin. Invest. 42#8:1300, 1963.

65. Shoemaker, W.C.; Yanof, H.M.; Turk, L.N., and Wilson, T.H.: Glucose and fructose absorption in the unanaesthetized dogs. Gastroenterology 44:654, 1963.

66. Wilson T.H., and Landau, B.R.: Specificity of sugar transport by the intestine of the hamster. Amer. J. Physiol. 198:99, 1960.

67. Cori, C.F.: The fate of sugar in animal body. I: The rate of absorption of hexoses and pentoses from the intestinal tract. J. Biol. Chem. 66:691, 1925.

68. Smith, L.H.; Ettinger, R.H., and Seligson, D.: A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. J. Clin. Invest. 32:273, 1953.

69. Weichselbaum, T.E.; Margraf, H.W., and Elman, R.: Metabolism of intravenously infused fructose in man. Metabolism 2:434, 1953.

70. Drury, D.R., and Wick , A.N.: Metabolism of mannose by the extra hepatic tissues. Amer. J. Physiol. 177:535, 1954.

71. Wyngaarden, J.B.; Segal, S., and Foley, J.B.: Physiological dispostion and metabolic fate of infused pentoses in man. J. Clin. Invest. 36:1395, 1957.

72. Czaky, T.T., and Lassen, U.V.: Active intestinal transport of D-xylose. Biochem, Biophys. Acta. 82:215, 1964.

73. Beaconsfield, P., and Ginsberg, J.: Hypoglycemia after oral ribose. Lancet ii:153, 1967, July 15.

74. Fisher, R.B., and Lindsay, D.B.: The action of insulin on the penetration of sugars into the perfused heart. J. Physiol. 131:526, 1956.

75. Helmreich, E., and Cori, C.F.: Studies of tissue permeability. II: The distribution of pentoses between plasma and muscle. J. Biol. Chem. 224:663, 1957.

76. Hiatt, H.H.: Glycogen formation via the pentose phosphate pathway in mice in vivo. J. Biol. Chem. 224:851, 1957.

77. Cori, C. F.: The rate of absorption of a mixture of glucose and galactose. Proc. Expt. Biol. Med. 23:290, 1926.

78. Williams, R. H.: Text Book of Endocrinology, W. B. Saunders Company Third Editions, 1965, p.p. 562.

79. Anderson, G. A.; Kologlu, Y., and Papadopoulos: Fluctuations in post absorptive blood glucose in relation to insulin release. Metabolism 16:586, 1967.

80. Allison S. P.; Prowse, K., and Chamberlain, M. J.: Failure of insulin response to glucose load during operation and myocardial infarction. Lancet 1:478, 1967, March 4.

81. Dixon W. J., and Massey, F. J. Jr.: Introduction to Statistical Analysis McGraw-Hill Book Company, 2nd Edition, 1957. p.p. 145-152.

SECTION VII

APPENDICES

APPENDIX A

METHODOLOGY

1. Hydrolysis of α-methyl Glucoside:

One millilitre of $Ba(OH)_2$-$ZnSO_4$ filterate was hydrolysed in 1 N HCl (1.0 ml. of 2N HCl) in a final volume of 2.0 mls, for two hours at 100°C. The hydrolysate was then cooled and 0,3 ml of 5 N NaOH solution was added to neutralize the acid partially. One ml. of 0.1 M phosphate buffer pH 7.40 was added to increase the buffering capacity and pH of the resulting solution was adjusted to 7.0 with sodium hydroxide solution. This solution was analysed for total reducing activity by the method of Nelson and Somogyi (57).

2. Preparation of Reagents:

i) Borate Buffer: Crystalline boric acid (8.25 grams) was dissolved in approximately 700-800 mls. of deionized distilled water and 2.70 grams of sodium hydroxide was added to the above solution while stirring. The pH of resulting solution was adjusted to 8.0 by adding drops of 12 N HCl. The final volume was made to a litre with further addition of water.

ii) Bovine Serum Albumin Solution (B.S.A.): Crystalline bovine serum albumin (Pentex Incorporated, Kankakee, Illinois) was added to appropriate volume of borate buffer and it was allowed to stand for two hours at 2-4°C in a refrigerator. The pH of the resulting solution was adjusted to 8.0 by adding drops of either 1 N HCl or 1 N NaOH. The final volume was made such that the final solution contained one gram of bovine serum albumin per one hundred mls. of solution. The pH of this solution was checked and the resultant solution was stored at 2-4°C in a refrigerator until its use in the assay. B.S.A. solution was

used for all purposes i.e. dilutions, preparation of standards, etc. in this immunoassay.

iii) Standards of Pork Insulin: Pork insulin was purchased from Cannaught Medical Research Laboratories, Toronto, Ontario, in solution form. One hundredth of one ml. of stock solution containing 80 units of insulin per ml. of the solution was diluted 1250 fold to give a solution containing 64,000 microunits (μU) of insulin per ml. The resulting solution was further diluted 2,000 fold to yield a solution containing 32 μUnits of insulin per ml. and by further dilutions, the solution containing 16, 8 and 4 μUnits per ml. were prepared. These standard solutions were divided into aliquots of one ml. and were frozen until the time of insulin assay. A set of standards i.e. 4, 8, 16 and 32 μUnits/ml. was thawed at room temperature and were analysed along with blanks, controls and serum samples as will be explained later.

iv) Anti insulin antibody-guinea pig (AIS-GP) Solution: These antibodies specific to pork insulin were obtained from immunized guinea pigs and were purchased from Pentex Incorporated in the crystalline form. One ml. of saline solution was to be added and mixed to give a solution of whole anti-insulin serum. Since it was found that freezing of this solution for two months was detrimental to its binding capacity to its antigen, it was considered suitable to dissolve a small amount of crystalline material in appropriate volume of saline and store the solution at 2-4^{o}C in a refrigerator rather than freezing. Since it was noted that the storage of the above solution in the manner explained above, for longer than a month, deteriorated its binding capacity with insulin, this solution was discarded after one month of its preparation and a fresh solution was prepared. The resultant

solution was diluted with B.S.A. seven thousands fold before its addition to the reaction mixture.

v) Insulin-I^{125} Solution: Solution containing 25,000 μUnits or 5 microcurrie radioactivity per ml. was purchased from the Radiochemical Centre, Amersham, England. This was diluted 16-21 fold with B.S.A. such that 0.1 ml. of the resultant solution when added to blanks would give 9 to 10 thousands counts per minute (c.p.m.).

vi) Purification of Resin: Amberlite-CG-400 was purchased from Rohm and Haas, Philadelphia, Pennsilvania and 25 grams of this resin was mixed with 200 mls. of 2 N NaOH solution for two hours. This was then filtred on a Buchner funnel and washed with distilled water repeatedly until the pH of the filterate was below 80. It was then dried at room temperature for two to three days and stored in an amber coloured bottle.

3. Immunoassay of Insulin:

i) Principle: Insulin binds to its specific antibody i.e. AIS-GP, forming a soluble complex, In the presence of labelled and unlabelled insulin (standards or serum sample) in the reaction mixture, both compete for this antibody and consequently, the binding between labelled insulin and antibody is decreased. This decrease in binding can be determined quantitatively by measuring the radioactivity of the complex.

ii) Serum Separation: The blood samples were let clot for 4-6 hours in a refrigerator at 2-4^{o} C and the serum was separated by centrifugation at 2,300 r.p.m. in a clinical centrifugation at 2,300 r.p.m. in a clinical centrifuge.

iii) Procedure: The assay of insulin consists of a two step reaction.

The method for the first step was followed from the immunoassay procedure of Soeldner and Slone (59). During this step, a reaction between insulin (both labelled and unlabelled) and its antibody was carried out for 24 hours at 2-4°C in one percent B.S.A., at pH 8.0. Appropriate blanks, controls and standards (in duplicates) were run along with each set of serum samples (in triplicate).

The second step of this reaction involves the separation of soluble complex and this was accomplished by a resin adsorption method of Meade et al (60). Purified resin (200 mgs.) was added to all the tubes except blanks and these tubes were shaken for sixty minutes at 100 cycles per minute at room temperature. The resin was separated by centrifugation at 2,300 r.p.m. for 20 minutes in a clinical centrifuge. One half ml. of the supernatant was withdrawn and its radioactivity was measured in a well type γ-scintillator. Each tube was counted three times and average c.p.m. were calculated and corrected for the background. Percent binding between insulin-I^{125} and antibodies were calculated by using the following formula.

$$\text{Percent binding} = \frac{\text{c.p.m. in supernatant of standard or serum sample}}{\text{Total counts per minute (blanks)}} \times 100$$

A standard curve (percent bound insulin-I^{125} versus insulin concentration in μUnits/ml) was plotted and the concentration of insulin in a serum sample was taken as that quantity of a standard concentration of insulin which would give the same percent binding as was obtained with serum sample. This insulin concentration of serum was corrected for the initial dilution factor of 5 fold.

iv) Recovery: A recovery sample in duplicate was run with each

new set of insulin standards. If the recovery percent was below seventy, the standards were considered unsatisfactory and new set of standards was prepared. This process was repeated until the recovery of added insulin was over seventy percent.

v) Immunoassay in Summary:

First step: The following additions were made to a reaction cell stepwise:

Reagents	Blank	Control	Standard	Serum Sample	Recovery
B.S.A. (1% pH 8.0)	1.10 mls.	1.00 ml.	--	0.80 ml.	0.50 ml.
Standards 4, 8, 16, 32 μU/ml.	--	--	1.00 ml.	--	0.40 ml. 4 μU/ml.
Serum Sample	--	--	--	0.20 ml.	0.10 ml.
AIS-GP (1:7000)	--	0.100 ml.	0.10 ml.	0.10 ml.	0.10 ml.
Insulin-I^{125} (10-16 μU/0.1 ml.)	0.10 ml.	0.10 ml.	0.10 ml.	0.10 ml.	0.10 ml.

The reactants were mixed gently and sealed with parafilm and incubated for 24 hours at 2-4°C in a refrigerator.

Second Step: Purified resin was added as follows:

Amberlite CG 400	--	200 mgs.	200 mgs.	200 mgs.	200 mgs.

The tubes were shaken for exactly one hour at the room temperature at a speed of approximately 100 cycles per minutes and were centrifuged in a

clinical centrifuge for 20 minutes at 2,300 r.p.m. One half ml. of the supernatant was placed in a counting test tube and the radioactivity was measured in γ-scintillator for three times and percent binding of insulin-I^{125} was calculated.

APPENDIX B

STATISTICAL ANALYSIS

1. Analysis of Variance (single classification)[1]:

The data analysed here, was taken from the intravenous saline experiments (Table 1).

The hypothesis tested was that the means of serum insulin levels at 0, 5, 15, 30, 60 and 120 minutes, after the intravenous infusion of saline, are equal.

$$\text{i.e. } H_0 = \bar{X}_{0'} = \bar{X}_{5'} = \bar{X}_{15'} = \bar{X}_{30'} = \bar{X}_{60'} = \bar{X}_{120'}.$$

The level of significance was chosen as 5 %.

$$\text{i.e. } \alpha = .05$$

The statistic used was F ratio

$$F = \frac{\text{mean square for means } (S_M^2)}{\text{mean square for within groups } (\bar{S}_p^2)}$$

It was assumed that these data were randomly selected from normal populations.

The critical value for F was 2.62.

$$\text{i.e. F crit. } F_{.95}(5, 24) = 2.62.$$

F ratio was, computed as follows:

	Sum of squares	df.	Mean square	F ratio
Category means	0.61	5	0.122	$F = \frac{0.122}{0.128} = 0.95$
Within groups	3.06	24	0.128	$F_{.95}(5, 24) = 2.62$
Total	3.67	29		

1. Dixon, W.J. and Massey, Jr. F.J. Introduction to Statistical Analysis (second edition McGraw-Hill Book Company, 1957) Page 145-152.

Since the computed F value was less than the critical value of 2.62, the hypothesis was accepted. This showed that the means of serum insulin level at different time intervals after the intravenous infusion of physiological saline, did not differ from one another at the 5% level of significance. i.e. $p > .05$.

Subsequently, an overall means control value was obtained by averaging six means at 0, 5, 15, 30, 60 and 120 minutes time intervals.

$$\text{i.e. } \bar{\bar{X}} = \frac{1.00 + 0.82 + 1.05 + 1.30 + 1.06 + 1.11}{6}$$

$$\text{S.E.} = .06$$

$$\bar{\bar{X}} \pm \text{S.E.} = 1.06 \pm 0.06$$

The data of the oral saline experiments (Table XIII) was treated exactly the same way and an overall mean control value of 0.99 ± 0.04 was obtained.

Each mean serum insulin level after the infusion of a test substance intravenously or orally, was compared with the respective overall mean control value and the significance of difference was determined by a t-test.

In order to show that the true blood glucose concentration was unchanged after the infusion of various test substances, the analysis of variance was used. p was always found to be greater than .05.

APPENDIX C

TABLES CONTAINING

EXPERIMENTAL DATA

TABLE I

Effects of Rapid Intravenous Infusion of Saline on Serum Insulin and Blood Glucose Concentration in Rabbits.

	Time in minutes after the infusion					
	0'		5'		15'	
Expt. No.	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level
163	86	1.00	87	1.10	85	1.75
164	110	1.00	110	1.01	112	0.96
166	108	1.00	120	0.76	100	0.75
174	78	1.00	78	0.44	85	0.83
175	103	1.00	110	0.80	103	0.95
$\bar{X}\pm$ S.E.	97± 6	1.00± -	101± 8	0.82± .11	97± 5	1.05± .18
p		-		-		-

TABLE I Continued

Effects of Rapid Intravenous Infusion of Saline on Serum Insulin and Blood Glucose Concentration in Rabbits

Time in minutes after the infusion					
30'		60'		120'	
Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Levels
77	1.08	79	1.20	79	1.88
147	1.86	206	0.96	120	0.98
110	1.51	98	1.24	121	0.86
78	1.20	72	1.31	78	1.37
113	0.85	144	0.57	112	0.46
105± 13	1.30± .18	120± 25	1.06± 0.14	102± 10	1.11± 0.24
	<.20		-		-

TABLE II

Effects of Intravenous Infusion of D(+) Glucose (1.5 gm. /3 cc's of solution/kg. b. wt.) on Serum Insulin and Blood Sugar Levels in Rabbits.

	Time in minutes after the infusion					
	0'		5'		15'	
Expt. No.	Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level
157	110	1.00	631	1.94	451	2.38
158	92	1.00	511	2.51	417	2.57
159	110	1.00	440	2.96	383	2.25
160	118	1.00	517	1.73	473	1.20
161	110	1.00	534	2.78	388	2.37
162	105	1.00	530	1.13	427	2.29
$\bar{X}\pm$ S.E.	108± 4	1.00± 0.00	527± 25	2.18± 0.29	423± 14	2.18± 0.20
p		-		$<.005$		$<.001$

* not significant

TABLE II Continued

Effects of Intravenous Infusion of D(+) Glucose (1.5 gm./3 cc's of solution/kg.b.wt.) on Serum Insulin and Blood Sugar Levels in Rabbits

Time in minutes after the infusion						
30'		60'		120'		
Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level	K(Glucose Disappearance Const.) $x10^{-2}$
295	1.04	171	1.91	99	1.30	7.16
289	2.41	161	1.90	24	0.50	5.95
273	2.50	81	2.14	0	1.46	5.80
299	1.39	215	0.79	102	0.94	4.86
226	2.25	108	1.16	23	1.09	7.38
248	2.22	136	1.97	20	1.26	7.12
271± 12	1.97± 0.25	145± 20	1.65± 0.22	46± 18	1.09± 0.14	6.38± 0.41
	$<.005$		$<.05$		N.S.*	

TABLE III

Effects of Intravenous Infusion of D(+) Mannose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
Expt. No.	Blood Mannose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Level	Blood Glucose Conc.	Serum Insulin Level
77	0	88	1.00	456	100	7.19	249	83	1.73
79	0	93	1.00	439	87	2.47	303	83	1.05
83	0	103	1.00	497	112	3.86	340	117	1.36
93	0	85	1.00	560	100	3.01	411	101	2.84
94	0	65	1.00	577	78	2.72	348	72	0.93
$\bar{X}\pm$ S.E.	-	87± 6	1.00± 0.00	506± 27	95± 6	3.85± 0.87	330± 27	91± 8	1.58± 0.34
p			-			$<.01$			$<.20$

* not significant

TABLE III Continued

Effects of Intravenous Infusion of D(+) Mannose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

Time in minutes after the infusion									
30'			60'			120'			
Blood Mannose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Level	Blood Glucose Conc.	Serum Insulin Level	K(Mannose Disappearance Const.) $x10^{-2}$
159	78	1.36	86	72	0.85	31	75	1.09	11.60
225	75	1.16	129	73	0.98	40	71	0.83	4.72
195	97	1.11	105	79	0.72	38	114	0.64	8.32
192	81	1.09	129	71	1.19	24	94	0.90	8.14
264	66	0.89	134	71	1.14	35	92	0.91	6.92
207± 18	79± 5	1.12± 0.08	117± 9	73± 2	0.98± 0.09	34± 3	89± 8	0.87± 0.07	7.94± 1.12
		N.S.*			N.S.*			N.S.*	

TABLE IV

Effects of Intravenous Administration of D-Fructose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin Levels and Blood Glucose Concentration in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
Expt. No.	Blood Fructose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Conc.	Blood Glucose Conc.	Serum Insulin Level
64	13	85	1.00	531	80	2.84	317	81	1.93
67	0	102	1.00	465	107	2.60	282	105	2.16
68	9	91	1.00	576	108	2.62	314	107	2.62
70	12	95	1.00	439	106	2.92	376	95	3.24
72	2	77	1.00	429	88	3.17	299	94	2.40
66	10	76	1.00	380	97	2.92	327	100	1.53
$\bar{X}\pm$ S.E.	8± 2	88± 4	1.00± 0.00	470± 29	98± 5	2.85± 0.09	319± 13	97± 4	2.31± 0.24
p			-			$<.001$			$<.001$

* not significant

TABLE IV Continued

Effect of Intravenous Administration of D-Fructose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin Levels and Blood Glucose Concentration in Rabbits

Time in minutes after the infusion									
30'			60'			120'			
Blood Fructose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Level	Blood Glucose Conc.	Serum Insulin Level	K(Fructose Disappearance Const.) $x10^{-2}$
199	72	1.44	91	70	1.56	27	90	1.22	13.12
149	89	1.40	66	79	1.23	21	102	0.88	12.60
202	91	1.38	66	94	1.31	32	158	0.80	14.24
204	80	2.16	38	85	2.11	43	106	0.89	10.44
202	91	1.90	101	107	1.17	22	112	0.93	8.92
135	92	2.00	90	73	1.53	31	85	1.62	9.52
182± 13	86± 3	1.71± 0.14	75± 10	85± 6	1.49± 0.14	29± 3	109± 11	1.06± 0.13	11.47± 0.88
		$<.005$			$<.025$			N.S.*	

TABLE V

Effects of Rapid Intravenous Injection of D(-) Ribose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits.

Time in minutes after the infusion

	0'			5'			15'		
Expt. No.	Blood Ribose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Level	Blood Glucose Conc.	Serum Insulin Level
133	0	100	1.00	505	95	2.03	344	105	1.80
134	0	100	1.00	-	-	1.53	366	184	1.30
145	-	87	1.00	491	85	1.34	317	88	1.95
146	0	80	1.00	478	85	1.56	318	81	1.65
147	0	77	1.00	459	92	1.63	391	82	1.71
$\bar{X}\pm$ S.E.	0	89± 5	1.00± 0.00	483± 10	89± 3	1.62± 0.11	347± 14	108± 20	1.68± 0.11
p			-			<.005			<.001

TABLE V Continued

Effects of Rapid Intravenous Injection of D(-) Ribose (1.5 gm./3cc's of solution/kg. b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

Time in minutes after the infusion

30'			60'			120'			
Blood Ribose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Level	Blood Glucose Conc.	Serum Insulin Level	K(Ribose Disappearance Const.) $x10^{-2}$
370	94	1.44	231	86	1.34	69	96	1.40	4.90
-	-	1.74	224	116	1.58	59	142	1.37	3.37
297	95	1.83	172	100	1.34	58	98	1.83	5.78
292	78	1.88	200	82	1.77	62	139	1.24	4.91
254	79	1.63	123	84	1.22	66	77	1.97	6.35
303± 24	87± 5	1.70± 0.08	190± 20	94± 6	1.45± 0.11	63± 2	110± 13	1.56± 0.14	5.06± 0.50
		$<.001$			$<.01$			$<.01$	

TABLE VI

Effect of Rapid Intravenous Infusion of D(+) Galactose (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
Expt. No.	Blood Galactose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Galactose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Galactose Level	Blood Glucose Conc.	Serum Insulin Level
123	0	86	1.00	486	86	1.54	398	81	1.16
124	0	65	1.00	501	71	1.33	415	71	0.92
125	0	87	1.00	504	89	1.78	436	85	1.66
168	0	90	1.00	490	93	1.80	362	97	1.65
169	0	90	1.00	529	93	1.37	428	90	1.67
170	0	103	1.00	474	122	2.26	392	120	1.34
$\bar{X}\pm$ S.E.	0	87± 5	1.00± 0.00	497± 8	92± 6	1.68± 0.14	405± 11	91± 6	1.40± 0.13
p			-			$<.005$			$<.05$

TABLE VI Continued

Effect of Rapid Intravenous Infusion of D(+) Galactose (1.5 gm./3cc's of solution/kg. b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

Time in minutes after the infusion									
30'			60'			120'			
Blood Galactose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Galactose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Galactose Level	Blood Glucose Conc.	Serum Insulin Level	K(Galactose Disappearance Const.) $x10^{-2}$
376	81	1.38	241	71	0.98	164	74	0.98	3.50
405	61	1.26	211	63	0.94	125	70	0.83	3.79
363	113	2.43	266	118	2.93	210	145	2.68	3.49
425	80	1.56	306	81	1.37	202	80	1.14	2.58
418	87	1.83	295	93	1.44	224	98	1.39	3.31
342	122	1.63	308	107	1.38	216	107	1.43	2.63
388± 14	91± 9	1.68± 0.17	271± 16	89± 8	1.51± 0.30	190± 16	96± 11	1.41± 0.27	3.22± 0.20
		<.01			<.20			<.40	

TABLE VII

Effects of Intravenous Infusion of D-Xylose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

Time in minutes after the infusion

	0'			5'			15'		
Expt. No.	Blood Xylose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Level	Blood Glucose Conc.	Serum Insulin Level
102	0	112	1.00	565	123	1.49	390	114	1.57
103	0	93	1.00	561	104	1.32	413	93	1.30
104	0	79	1.00	479	88	1.68	361	87	1.19
105	0	85	1.00	488	102	1.12	367	86	1.68
106	0	93	1.00	525	112	1.81	398	95	1.48
107	0	86	1.00	510	90	1.18	460	87	1.20
$\bar{X}\pm$	0±	91±	1.00±	521±	103±	1.43±	398±	94±	1.40±
S.E.	0	5	0.00	15	5	0.11	15	4	0.08
p			-			<.025			<.010

TABLE VII Continued

Effects of Intravenous Infusion of D-Xylose (1.5 gm./3cc's of solution/kg. b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

Time in minutes after the infusion									
30'			60'			120'			
Blood Xylose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Level	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Level	Blood Glucose Conc.	Serum Insulin Level	K(Xylose Disappearance Const.) $x10^{-2}$
329	103	2.06	245	83	0.55	114	103	0.82	3.24
309	86	2.47	221	74	1.18	157	91	1.76	4.16
286	78	1.31	175	79	1.15	105	79	1.27	4.30
274	85	1.10	168	81	1.01	106	107	0.79	4.86
312	103	2.13	204	91	1.07	117	98	0.90	4.15
352	100	1.80	253	97	1.20	150	169	2.30	3.74
310± 12	93± 4	1.81± 0.21	211± 14	84± 3	1.03± 0.10	125± 9	108± 13	1.31± 0.25	4.08± 0.22
		<.01			N.S.*			<.40	

* not significant

TABLE VIII

Effect of Rapid Intravenous Infusion of α-Methyl Glucoside (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
Expt. No.	α-CH_3-glucoside Level	Blood Glucose Conc.	Serum Insulin Level	α-CH_3-glucoside Level	Blood Glucose Conc.	Serum Insulin Level	α-CH_3-glucoside Level	Blood Glucose Conc.	Serum Insulin Level
111	0	70	1.00	927	60	0.65	746	67	0.59
112	0	94	1.00	1,058	82	0.85	1,045	85	-
119	0	81	1.00	901	67	0.90	818	57	0.97
120	0	85	1.00	737	83	0.60	730	84	1.43
121	0	89	1.00	776	77	1.10	644	79	1.17
122	0	85	1.00	693	79	0.91	547	79	0.85
138	0	70	1.00	774	56	0.96	938	62	1.40
139	0	77	1.00	676	82	1.12	-	-	1.37
140	0	70	1.00	-	-	-	703	70	0.91
$\bar{X}\pm$ S.E.	0	80± 3	1.00± 0.00	818± 47	73± 4	0.89± 0.07	784± 63	73± 4	1.08± .11
p			-			N.S.*			N.S.*

* not significant

TABLE VIII Continued

Effect of Rapid Intravenous Infusion of α-Methyl Glucoside (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

Time in minutes after the infusion									
30'			60'			120'			
α-CH_3-glucoside Level	Blood Glucose Conc.	Serum Insulin Level	α-CH_3-glucoside Level	Blood Glucose Conc.	Serum Insulin Level	α-CH_3-glucoside Level	Blood Glucose Conc.	Serum Insulin Level	K(α-CH_3-Glucoside Disappearance (Const.) $x10^{-2}$
614	71	0.51	516	73	1.19	402	77	0.85	6.27
807	87	0.73	811	94	1.00	449	98	0.93	4.51
736	73	0.51	-	-	-	492	78	0.92	4.54
696	81	1.30	434	80	3.60	451	72	0.97	2.57
601	79	0.69	464	75	0.67	447	71	0.75	4.65
509	74	0.85	417	69	1.00	365	66	0.92	3.90
521	69	1.10	471	71	1.24	405	86	0.86	4.42
490	69	1.14	444	66	0.27	-	-	0.98	4.39
569	66	0.91	464	80	1.23	361	72	1.49	4.38
616± 37	74± 2	0.86± .09	503± 45	76± 3	1.28± .35	422± 16	78± 4	0.96± .07	4.40± 0.32
		N.S.*			N.S.*			N.S.*	

TABLE IX

Effect of Intravenous Infusion of Pyruvate (Na+) over 5 mintues (0.91 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

	Time in minutes after the infusion					
	0'		5'		15'	
Expt. No.	Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level
178	-	1.00	-	1.56	-	1.83
185	76	1.00	81	1.38	91	1.34
186	91	1.00	94	1.05	83	1.21
187	73	1.00	73	0.84	70	0.99
188	133	1.00	106	1.17	105	1.28
$\bar{X}\pm$	93±	1.00±	89±	1.20±	87±	1.33±
S.E.	14	0.00	7	0.13	7	0.14
p		-		$<.40$		$<.10$

TABLE IX Continued

Effect of Intravenous Infusion of Pyruvate (Na+) over 5 mintues (0.91 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

Time in minutes after the infusion					
30'		60'		120'	
Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level	Blood Glucose Conc.	Serum Insulin Level
-	1.87	-	1.74	-	1.65
91	2.08	116	2.30	110	1.59
95	1.06	99	1.66	133	1.05
84	1.00	93	1.25	87	1.17
110	1.54	95	1.18	139	1.00
95± 6	1.51± 0.21	101± 5	1.63± 0.20	117± 12	1.29± 0.14
	<.05		<.025		<.20

TABLE X

Comparison of Decay Constant (K) of Various Monosaccharides Infused Intravenously*

Sugar Infused	Glucose		Mannose		Fructose	
Glucose	$6.38 \pm 0.41 \times 10^{-2}$					
1x) Mannose	≈	$p < .10$	$7.94 \pm 1.12 \times 10^{-2}$			
2t) Fructose	>>>>>	$< .001$	>	$p < .05$	$11.47 \pm 0.88 \times 10^{-2}$	
Galactose	<<<<<	$< .001$	<<<<	$< .005$	<<<<<	$p < .001$
Xylose	<<<<<	$< .001$	<<<<	$< .005$	<<<<<	$< .001$
Ribose	≈	$< .10$	<	$< .05$	<<<<<	$< .001$
∝-methyl glucoside	<<<<<	$< .001$	<<<<	$< .005$	<<<<<	$< .001$

* Read from left to right as follows:

1x) Mannose was removed from the circulation at approximately the same rate as glucose.

2t) Fructose was removed from the circulation at a rate much faster than glucose.

>>>>> much faster.

> little faster.

TABLE X Continued

Comparison of Decay Constant (K) of Various Monosaccharides Infused Intravenously*

Galactose		Xylose		Ribose		∝methyl glucoside
$3.22 \pm 0.20 \times 10^{-2}$						
>	$p < .02$	$4.08 \pm 0.22 \times 10^{-2}$				
>>>	$< .01$	≋	$p < .10$	$5.06 \pm 0.50 \times 10^{-2}$		
>	$< .02$	≡	$< .50$	=	$p < .30$	$4.40 \pm 0.32 \times 10^{-2}$

≋ approximately same.

< little slower.

<<<< much slower.

= equal to.

≡ equivalent to.

TABLE XI

Comparison of maximum increases in Serum Insulin Levels after the Intravenous Infusion of Different Monosaccharides*

;ar Infused	Saline	Glucose	Mannose	Fructose
ine	1.06±0.06			
ıcose**	>>>>> p<.001	2.18±0.20		
nnose	>>> <.01	≈p <.10	3.85±0.87	
uctose	>>>>> <.001	>> <.02	= p<.30	2.85±0.09
lactose	>>>> <.005	≈ <.10	<< <.025	<<<<< p<.001
lose	>>> <.01	= <.30	< <.05	<<<< <.005
ɔose	>>>> <.001	≈ <.10	< <.05	<<<<< <.001
-methyl coside	≡ <.70	≈ <.10	<<< <.01	<<<< <.005
ruvate	>> <.025	≈ <.10	< <.05	<<<<< <.001

Read from left to right as follows:

Maximum rise in S.I.L. after glucose was (>>>>>) much greater than after saline.

>> greater.

≈ approximately equal

TABLE XI Continued

Comparison of maximum increases in Serum Insulin Levels after the Intravenous Infusion of Different Monosaccharides*

Galactose	Xylose	Ribose	∝-methyl glucoside	Pyruvate
1.68±0.14				
≡ p <.70	1.81±0.21			
≡ <.90	≡ p <.70	1.70±0.08		
= <.40	= <.30	= p <.40	1.28±0.35	
≡ <.90	≡ <.60	≡ <.80	≡ <.50	1.63±0.20

= equal.

< lower - or less.

≡ equivalent.

TABLE XII

Effects of Rapid Intraduodenal Administration of D(+) Glucose (400 mgs./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

	Time in minutes after the infusion					
	0'		5'		15'	
Expt. No.	Rise in Blood Sugar Conc.	Serum In-sulin Level	Rise in Blood Sugar Conc.	Serum In-sulin Level	Rise in Blood Sugar Conc.	Serum In-sulin Level
12	0	1.00	-	-	2	0.77
20	0	1.00	-	-	58	0.20
21	0	1.00	-	-	35	0.63
23	0	1.00	-	-	25	1.03
24	0	1.00	-	-	133	0.89
29	0	1.00	-	-	30	0.43
32	0	1.00	-	-	37	0.96
30	0	1.00	-	-	1	0.81
34	0	1.00	-	-	67	1.45
35	0	1.00	-	-	57	1.11
$\bar{X}\pm$	0	1.00±			44±	0.83±
S.E.		0.00			12	.11
p*	-	-			$<.70$	$<.005$

* Values are obtained by comparing Blood Sugar Levels as well as Serum Insulin Levels after the oral administration of D(+) Glucose with those after the intraduodenal administration of D(+) Glucose.

TABLE XII Continued

Effects of Rapid Intraduodenal Administration of D(+) Glucose (400 mgs./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Levels in Rabbits

Time in minutes after the infusion					
30'		60'		120'	
Rise in Blood Sugar Conc.	Serum In-sulin Level	Rise in Blood Sugar Conc.	Serum In-sulin Level	Rise in Blood Sugar Conc.	Serum In-sulin Level
2	0.73	35	0.87	20	0.89
110	0.20	133	0.80	100	0.41
94	0.54	38	0.55	155	0.56
62	1.09	131	0.91	49	1.32
14	1.19	4	0.83	19	1.33
36	0.38	42	0.31	57	0.80
87	0.93	52	0.89	110	0.84
24	0.65	68	0.89	124	0.81
109	0.50	150	-	90	1.33
65	0.79	161	0.83	71	1.39
60± 13	0.70 .10	82± 18	0.76± .07	80± 14	0.97± .11
<.60	<.001	<.90	<.001	<.90	<.40

TABLE XIII

Effects of Rapid Administration of Physiological Saline (3cc's of saline/kg. b. wt.) Intragastrically (Orally) on Serum Insulin and Blood Glucose Concentrations in Rabbits

	Time in minutes after the infusion					
	0'		5'		15'	
Expt. No.	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level
165	79	1.00	79	1.00	76	0.75
167	98	1.00	98	1.23	105	0.83
168	86	1.00	96	1.72	86	0.97
176	89	1.00	88	0.97	101	1.21
177	87	1.00	88	0.95	85	1.07
$\bar{X}\pm$ S.E.	88± 3	1.00± 0.00	90± 4	1.17± 0.15	91± 5	0.97± 0.08
p						

TABLE XIII Continued

Effects of Rapid Administration of Physiological Saline (3cc's of saline/kg. b. wt.) Intragastrically (Orally) on Serum Insulin and Blood Glucose Concentrations in Rabbits

Time in minutes after the infusion

30'		60'		120'	
Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level
84	0.80	117	1.16	100	0.86
144	1.04	153	0.84	120	0.79
86	1.00	98	0.83	109	0.70
90	0.86	101	1.18	99	1.08
81	1.19	85	0.84	96	0.82
97± 12	0.98± 0.07	111± 12	0.97± 0.08	105± 4	0.85± 0.06

TABLE XIV

Effects of Rapid Oral Administration of D(+) Glucose (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

	Time in minutes after the infusion						
	0'			5'		15'	
Expt. No.	Total Reducing Sugars	Rise in Blood Glucose Conc.	Serum Insulin Level	Rise in Blood Glucose Conc.	Serum Insulin Level	Rise in Blood Glucose Conc.	Serum Insulin Level
180	94	0	1.00	12	1.05	35	1.31
181	96	0	1.00	21	1.04	82	1.52
182	84	0	-	10	-	33	-
183	84	0	1.00	20	2.28	41	1.54
184	76	0	1.00	0	1.59	8	1.35
191	88	0	1.00	0	1.43	33	2.54
192	110	0	1.00	0	1.24	20	1.44
$\bar{X}\pm$	90±	0	1.00±	9±	1.44±	36±	1.62±
S.E.	4		0.00	3	0.19	9	17
p			-		<.10		<.025

TABLE XIV Continued

Effects of Rapid Oral Administration of D(+) Glucose (1.5 gm./3cc's of solution/kg. b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

Time in minutes after the infusion					
30'		60'		120'	
Rise in Blood Glucose Conc.	Serum In-sulin Level	Rise in Blood Glucose Conc.	Serum In-sulin Level	Rise in Blood Glucose Conc.	Serum In-sulin Level
43	1.45	53	0.99	43	1.02
134	2.47	166	1.30	146	0.83
69	-	106	-	87	-
100	1.77	105	2.06	107	1.78
6	1.47	10	1.59	17	1.00
76	3.09	76	1.88	65	1.06
70	1.64	88	1.59	100	1.25
71± 15	1.98± 0.27	86± 18	1.57± 0.16	81± 16	1.16± 0.14
	$<.010$		$<.025$		$<.40$

TABLE XV

Effects of Oral Administration of D(+) Mannose Rapidly (1.5gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
pt.	Blood Mannose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Conc.	Blood Glucose Conc.	Serum Insulin Level
	0	80	1.00	8	77	1.03	16	81	1.66
	0	81	1.00	9	89	1.64	10	90	1.62
	0	98	1.00	0	100	1.01	0	99	1.63
	0	100	1.00	16	92	1.19	4	92	1.22
	0	77	1.00	7	77	1.05	5	76	1.80
	0	87±	1.00±	8±	87±	1.18±	7±	88±	1.58±
E.		5	0.00	3	5	0.12	3	4	0.10
			-			<.20			<.001

TABLE XV Continued

Effects of Oral Administration of D(+) Mannose Rapidly (1.5 gm./3cc's of solution/kg. b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

Time in minutes after the infusion								
30'			60'			120'		
Blood Mannose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Mannose Conc.	Blood Glucose Conc.	Serum Insulin Level
10	80	2.41	6	72	1.56	24	71	1.09
17	91	1.64	28	84	1.69	36	77	1.60
7	103	1.72	16	101	1.47	20	97	1.45
5	92	1.29	12	84	1.90	22	79	1.53
4	77	1.54	4	81	1.47	23	88	1.47
9± 2	89± 5	1.72± 0.19	13± 4	84± 5	1.62± 0.08	25± 3	82± 5	1.43± 0.09
		<.01			<.001			<.005

TABLE XVI

Effects of Oral Infusion of D-Fructose (1.5 gm./3cc's of solution/kg.b.wt.) Rapidly on Serum Insulin and Blood Glucose Concentrations in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
pt.	Blood Fructose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Conc.	Blood Glucose Conc.	Serum Insulin Level
	8	90	1.00	13	85	0.83	12	86	1.34
	3	98	1.00	6	95	0.90	5	107	1.28
	1	97	1.00	8	90	1.39	14	86	1.35
	13	99	1.00	0	91	1.87	1	91	1.91
	8	71	1.00	11	77	1.20	12	100	1.40
± E.	7± 2	91± 5	1.00±	8± 2	88± 3	1.24± 0.19	9± 3	94± 4	1.46± 0.12
			-			<.40			<.01

TABLE XVI Continued

Effect of Oral Infusion of D-Fructose (1.5 gm./3cc's of solution/kg.b.wt.) Rapidly on Serum Insulin and Blood Glucose Concentrations in Rabbits

Time in minutes after the infusion								
30'			60'			120'		
Blood Fructose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Fructose Conc.	Blood Glucose Conc.	Serum Insulin Level
0	104	1.25	15	81	1.73	6	92	1.28
14	111	1.10	17	132	1.74	13	158	1.52
14	86	1.33	12	79	1.41	13	85	1.39
3	87	1.27	43	91	1.77	7	94	1.96
3	89	1.57	5	74	1.87	2	99	1.87
7± 3	95± 5	1.30± 0.08	18± 7	91± 11	1.70± 0.08	8± 2	106± 13	1.60± 0.13
		<.01			<.001			<.005

TABLE XVII

Effect of Rapid Oral Administration of D(-) Ribose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
xpt. o.	Blood Ribose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Conc.	Blood Glucose Conc.	Serum Insulin Level
26	0	97	1.00	0	91	1.03	43	90	1.47
27	0	86	1.00	0	64	1.11	27	70	1.76
28	0	84	1.00	0	69	1.56	5	86	1.68
29	0	78	1.00	0	86	-	48	112	1.04
30	0	84	1.00	0	84	1.22	35	84	1.54
31	0	100	1.00	0	76	1.13	3	77	1.30
± . E.	0	88± 4	1.00± 0.00	0	78± 4	1.21± 0.09	27± 8	87± 6	1.47± 0.11
			-			<.10			<.005

TABLE XVII Continued

Effect of Rapid Oral Administration of D(-) Ribose (1.5 gm./ 3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

Time in minutes after the infusion								
30'			60'			120'		
Blood Ribose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Ribose Conc.	Blood Glucose Conc.	Serum Insulin Level
45	88	1.50	68	87	1.86	54	79	1.50
9	81	1.14	31	72	1.03	40	67	1.00
17	79	1.74	59	90	1.70	41	83	1.65
84	90	1.48	114	140	1.60	83	110	1.20
45	87	1.11	88	81	1.22	52	86	1.16
9	78	1.11	53	81	1.22	33	84	1.19
35± 12	84± 2	1.35± 0.11	69± 12	92± 10	1.44± 0.13	51± 7	85± 6	1.28± 0.10
		<.025			<.025			<.05

TABLE XVIII

Effect of Rapid Oral Administration of D(+) Galactose (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentration in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
xpt. o.	Blood Galac-tose Conc.	Blood Glu-cose Conc.	Serum In-sulin Level	Blood Galac-tose Conc.	Blood Glu-cose Conc.	Serum In-sulin Level	Blood Galac-tose Conc.	Blood Glu-cose Conc.	Serum In-sulin Level
76	0	97	1.00	2	97	1.11	38	91	1.96
81	0	84	1.00	7	85	1.37	29	84	1.42
85	0	88	1.00	3	97	0.82	45	85	1.39
89	0	93	1.00	5	97	1.31	58	97	2.09
90	0	83	1.00	0	82	1.37	19	83	1.76
91	0	90	1.00	0	92	0.81	81	94	1.45
92	0	78	1.00	11	78	0.81	40	68	1.12
±	0	88±	1.00±	4±	90±	1.09±	44±	86±	1.60±
. E.		2	0.00	2	3	0.10	8	4	0.13
			-			<.50			<.005

TABLE XVIII Continued

Effect of Rapid Oral Administration of D(+) Galactose (1.5 gm./3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentration in Rabbits

Time in minutes after the infusion								
30'			60'			120'		
Blood Galactose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Galactose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Galactose Conc.	Blood Glucose Conc.	Serum Insulin Level
88	90	2.23	190	141	2.56	149	150	1.67
80	76	1.20	108	77	2.37	168	81	2.10
50	87	1.82	37	84	2.48	116	77	3.25
92	97	1.54	162	193	2.09	-	-	-
107	83	1.82	115	83	1.70	137	81	1.36
61	90	2.73	113	89	1.17	134	81	1.10
83	79	1.45	85	67	1.02	169	69	1.02
80± 7	86± 3	1.83± 0.20	115± 19	105± 17	1.91± 0.24	146± 9	90± 12	1.75± 0.34
		<.01			<.01			<.10

TABLE XIX

Effect of Rapid Oral Administration of D-Xylose (1.5 gm. /3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentration in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
Expt. No.	Blood Xylose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Conc.	Blood Glucose Conc.	Serum Insulin Level
99	0	89	1.00	11	75	0.73	57	85	1.50
00	0	68	1.00	2	64	1.04	28	71	1.70
01	0	89	1.00	5	80	1.20	40	81	1.67
08	0	90	1.00	23	93	1.37	58	92	1.02
09	0	89	1.00	7	90	1.18	30	99	1.78
10	0	99	1.00	17	90	1.39	54	100	1.67
48	0	94	1.00	28	92	1.65	48	88	1.70
149	0	93	1.00	11	97	1.00	29	104	1.67
150	0	94	1.00	23	99	1.13	55	103	1.08
$\bar{X}\pm$ S.E.	0	89± 3	1.00± 0.00	14± 3	87± 4	1.19± .09	44± 4	91± 4	1.53± 0.09
p			-			<.20			<.005

TABLE XIX Continued

Effect of Rapid Oral Administration of D-Xylose (1.5 gm. /3cc's of solution/kg.b.wt.) on Serum Insulin and Blood Glucose Concentration in Rabbits

Time in minutes after the infusion								
30'			60'			120'		
Blood Xylose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood Xylose Conc.	Blood Glucose Conc.	Serum Insulin Level
81	88	1.64	103	81	0.87	90	85	1.54
73	71	1.70	80	71	1.80	103	81	1.72
45	93	1.64	71	91	1.51	115	97	1.67
124	130	1.35	136	154	1.64	112	89	1.05
108	99	1.33	147	105	1.36	157	105	1.36
77	92	1.47	118	83	1.40	127	92	1.33
61	91	1.28	50	97	-	79	87	0.91
33	114	1.58	56	87	1.73	68	96	1.61
89	97	0.96	127	82	0.95	77	77	1.05
77± 10	97± 7	1.44± .08	99± 12	95± 8	1.41± 0.12	103± 9	90± 3	1.36± 0.10
		<.005			<.05			<.025

TABLE XX

Effects of Oral Infusion of ∝-methyl Glucoside Rapidly (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

	Time in minutes after the infusion								
	0'			5'			15'		
Expt. No.	Blood ∝-CH_3-glucoside Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood ∝-CH_3-glucoside Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood ∝-CH_3-glucoside Conc.	Blood Glucose Conc.	Serum Insulin Level
113	0	78	1.00	0	78	0.93	96	75	1.00
114	0	92	1.00	0	92	0.91	44	87	1.33
115	0	70	1.00	0	71	0.79	168	67	2.64
116	0	83	1.00	0	84	0.98	55	86	1.11
118	0	100	1.00	0	100	0.74	148	104	1.21
141	0	105	1.00	0	103	1.12	52	111	1.02
142	0	92	1.00	0	83	1.16	-	94	1.04
143	0	86	1.00	0	80	1.06	-	81	1.01
144	0	72	1.00	0	67	1.06	-	70	1.06
$\bar{X}\pm$ S. E.	0	86± 4	1.00± -	0	84± 4	0.97± 0.05	94± 22	86± 5	1.27± 0.18
p			-			N.S.*			<.20

* not significant

TABLE XX Continued

Effects of Oral Infusion of α-methyl Glucoside Rapidly (1.5 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Glucose Concentrations in Rabbits

Time in minutes after the infusion								
30'			60'			120'		
Blood α-CH_3-glucoside Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood α-CH_3-glucoside Conc.	Blood Glucose Conc.	Serum Insulin Level	Blood α-CH_3-glucoside Conc.	Blood Glucose Conc.	Serum Insulin Level
277	84	1.06	371	84	0.81	514	89	1.35
-	92	0.64	162	94	0.69	140	101	0.39
249	95	2.05	351	71	1.15	502	103	1.05
188	90	1.05	269	91	1.22	276	117	1.08
333	107	1.21	442	118	1.09	483	100	0.94
211	77	0.88	280	103	0.85	182	105	1.09
-	96	1.21	-	86	0.99	315	80	0.97
120	80	1.02	320	76	1.11	232	87	1.02
-	68	1.26	146	70	1.30	260	72	1.27
230± 30	88± 4	1.15± 0.13	293± 36	88± 5	1.02± 0.07	320± 49	95± 5	1.02± 0.09
		<.40			N.S.*			N.S.*

TABLE XXI

Effects of Rapid Oral Administration of Glycine (Equimoles 0.62 gm./3cc's of solution/kg. b. wt.) on Serum Insulin and Blood Sugar Levels in Rabbits

	Time in minutes after the infusion					
Expt. No.	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level
171	90	1.00	94	0.78	94	1.36
172	69	1.00	68	1.01	69	1.06
173	76	1.00	71	1.13	105	1.68
189	80	1.00	-	-	89	1.07
190	91	1.00	91	0.80	89	0.92
$\bar{X} \pm$ S.E.	81± 4	1.00± 0.00	81± 7	0.93± 0.09	89± 6	1.22± 0.14
p		-		N.S.*		<.20

* not significant

TABLE XXI Continued

Effects of Rapid Oral Administration of Glycine (Equimoles 0.62 gm./3cc's of solution/kg. b.wt.) on Serum Insulin and Blood Sugar Levels in Rabbits

Time in minutes after the infusion					
30'		60'		120'	
Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level	Total Reducing Sugars	Serum Insulin Level
107	2.33	96	0.78	96	0.70
69	0.87	78	0.91	78	1.12
129	1.88	106	1.77	75	1.27
91	1.09	83	1.04	83	1.01
100	1.06	108	1.00	100	1.03
99±	1.45±	94±	1.10±	86±	1.03±
10	0.28	6	0.17	5	0.09
	<.20		N.S.*		N.S.*

TABLE XXII

Comparison of Maximum Increases in Serum Insulin Levels (Intravenous versus Oral) and in Actual Blood Sugar Concentration (Intravenous versus Oral).

Substance Infused	Route of Infusion	Max. Rise in Actual Blood Sugar Level $\bar{X}\pm$S. E.	Max. Rise in Serum Insulin Level $\bar{X}\pm$X. E.
Saline (Physiological)	I. V.	5±4	1.30±0.18
	Oral	17±3	1.17±0.15
Glucose	I. V.	527±25	2.18±0.20
	Oral	86±18	1.98±0.27
Mannose	I. V.	506±27	3.85±0.87
	Oral	25±3	1.72±0.19
Fructose	I. V.	470±29	2.85±0.09
	Oral	18±7	1.70±0.08
Galactose	I. V.	497±8	1.68±0.14
	Oral	146±9	1.91±0.24
α-CH_3-Glucoside	I. V.	818±47	1.27±0.35
	Oral	320±49	1.27±0.18
Xylose	I. V.	521±15	1.81±0.21
	Oral	103±9	1.58±0.08
Ribose	I. V.	483±10	1.70±0.08
	Oral	69±12	1.47±0.11
Glucose	Intraduodenal	82±18	0.70±0.10
	Oral	86±18	1.98±0.27

* The significance of differences between the maximum rises in actual blood sugar concentration after the intravenous and the oral infusions of different monosaccharides.

TABLE XXII Continued

Comparison of Maximum Increases in Serum Insulin Levels (Intravenous versus Oral) and in Actual Blood Sugar Concentration (Intravenous versus Oral)

p 1*	p 2**
<.001 I.V. >>>>> Oral	<.60 I.V. ≡ Oral
<.001 I.V. >>>>> Oral	<.05 I.V. > Oral
<.001 I.V. >>>>> Oral	<.001 I.V. >>>>> Oral
<.001 I.V. >>>>> Oral	<.50 I.V. ≡ Oral
<.001 I.V. >>>>> Oral	<.90 I.V. ≡ Oral
<.001 I.V. >>>>> Oral	<.20 I.V. = Oral
<.001 I.V. >>>>> Oral	<.20 I.V. = Oral
N.S. Intraduodenal ≡ Oral	<.001 Intraduodenal <<<<< Oral

**The significance of difference between the maximum rises in serum insulin levels after the intravenous and the oral infusions of different monosaccharides.

TABLE XXIII

Comparison of Present Findings With the Previously Reported Data

;st bstance	Experi-mental Conditions	Subject of Study	Index of Insulin	Insulin Secretion	Authors (Ref. #)	Type of Insulin Response
	Intravenous	Man	IRI	+	Karam et al.(8)	
		Sheep	IRI	+	Boda (7)	
		Dog	IRI	+	Goetz et al.(11)	
		Rabbit	IRI	+	Present Findings	Immediate, marked and sustained
.ucose	In Vitro	Rat	IRI	+	Malaisse et al.(13)	
		Rabbit	IRI	+	Coore et al.(12)	
	Perfusion	Rat	IRI	+	Grodsky et al.(9)	
		Rat	IRI	+	Sussman et al.(10)	
	Cross - Circulation	Dog	Hypogly-cemia	+	Pozza et al.(6)	
	Intravenous	Man	IRI	++	Karam et al.(8)	
		Man	ILA	-	Sheps et al.(61)	
		Rabbit	IRI	++	Present Findings	immediate, marked and very transient
Iannose	In Vitro	Rabbit	IRI	+	Coore et al.(12)	
		Rat	IRI	+	Malaisse et al.(13)	
	Perfusion	Rat	IRI	+	Grodsky et al.(9)	
		Rat	IRI	+	Sussman et al.(10)	
	Cross-Circulation	Dog	Hypogly-cemia	-	Pozza et al.(6)	

TABLE XXIII Continued

Comparison of Present Findings With the Previously Reported Data

Test Substance	Experimental Conditions	Subject of Study	Index of Insulin	Insulin Secretion	Authors (Ref. #)	Type of Insulin Response
	Intravenous	Sheep	IRI	+	Boda et al.(7)	
		Rabbit	IRI	+	Present Findings -	immediate, marked and unsustained
Fructose	In Vitro	Rat	IRI	-	Malaisse et al.(13)	
		Dog	IRI	+	Goetz et al.(11)	
		Rabbit	IRI	-	Coore et al.(12)	
	Perfusion	Rat	IRI	+	Grodsky et al.(9)	
		Rat	IRI	+	Sussman et al.(10)	
	Cross-Circulation	Dog	Hypoglycemia	-	Pozza et al.(6)	
	Intravenous	Dog	IRI	+	Goetz et al.(11)	
		Rabbit	IRI	+	Present Findings-	delayed, moderate and sustained
Ribose	In Vitro	Rabbit	IRI	-	Coore et al.(12)	
		Rat	IRI	-	Malaisse et al.(13)	
	Cross-Circulation	Dog	Hypoglycemia	+	Pozza et al.(6)	

Comparison of Present Findings With the Previously Reported Data

Test Substance	Experimental Conditions	Subject of Study	Index of Insulin	Insulin Secretion	Authors (Ref. #)	Type of Insulin Response
Galactose	Intravenous	Man	IRI	-	Karam et al.(8)	
		Dog	IRI	+	Goetz et al.(11)	
		Sheep	IRI	+	Boda (7)	
		Rabbit	IRI	+	Present Findings-	delayed, moderate and sustained
	In Vitro	Rabbit	IRI	-	Coore et al.(12)	
		Rat	IRI	-	Malaisse et al.(13)	
	Perfusion	Rat	IRI	-	Grodsky et al.(9)	
		Rat	IRI	-	Sussman et al.(10)	
	Cross-Circulation	Dog	Hypoglycemia	+	Pozza et al.(6)	
Xylose	Intravenous	Rabbit	IRI	+	Present Findings-	delayed, moderate and sustained
	In Vitro	Rabbit	IRI	-	Coore et al.(12)	
		Rat	IRI	-	Malaisse et al.(13)	
	Perfusion	Rat	IRI	-	Grodsky et al.(9)	
	Cross-Circulation	Dog	Hypoglycemia	-	Pozza et al.(6)	
α-methyl Glucoside	Intravenous	Rabbit	IRI	-	Present Findings	No response

TABLE XXIII Continued

Comparison of Present Findings With the Previously Reported Data

Test Substance	Experimental Conditions	Subject of Index	Index of Insulin	Insulin Solution	Authors (Ref. #)	Type of Insulin Response
	Intravenous	Rabbit	IRI	+	Present Findings	delayed and slight
Pyruvate	In Vitro	Rabbit	IRI	-	Coore et al (12)	
	Perfusion	Rat	IRI	+	Grodsky et al (9)	