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EXPERIMENTAL DIABETES IN THE BABOON

THESIS

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THIS WHOLE THESIS IS THE CANDIDATE'S OWN ORIGINAL WORK.

## CONTENTS.

	PAGE
ACKNOWLEDGEMENTS .....	1
CHAPTER ONE : INTRODUCTION .....	2
CHAPTER TWO : DRUG INDUCED DIABETES IN ANIMALS .....	10
CHAPTER THREE : MATERIALS - METHODS - TECHNIQUES .....	20
CHAPTER FOUR : STREPTOZOTOCIN DIABETES IN THE BABOON .....	29
CHAPTER FIVE : GLUCOSE KINETICS .....	35
CHAPTER SIX : LACTATE KINETICS .....	63
CHAPTER SEVEN : FOREARM METABOLISM .....	82
CHAPTER EIGHT : GLUCOSE AND LACTATE INTERRELATIONSHIPS .	113
SUMMARY .....	119
APPENDIX ONE .....	121
APPENDIX TWO .....	123
APPENDIX THREE .....	125
APPENDIX FOUR .....	126
APPENDIX FIVE .....	131
APPENDIX SIX .....	133
APPENDIX SEVEN .....	135
REFERENCES .....	137

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## CHAPTER ONE

## INTRODUCTION

Claude Bernard (1813-1878) proposed the theory of glucose formation from hepatic glycogen and suggested that the high blood glucose level in diabetes mellitus might be due to overproduction of glucose by the liver. Thus over a century ago abnormal hepatic metabolism in diabetes mellitus (hereafter referred to as diabetes) was proposed. In the first half of this century interest centred mainly on the study of peripheral glucose metabolism and the role of the liver received little attention. The discovery of insulin by Banting and Best in 1922 was a major stimulus to the study of peripheral glucose metabolism. In the past two decades, however, new interest in the role of the liver in diabetes has arisen. The development of improved radioactive labelling and counting stimulated the use of isotopic techniques to measure hepatic glucose production rates. An appreciation of the role of hepatic and peripheral abnormalities in the pathogenesis of hyperglycaemia in diabetes slowly evolved.

"The advance of knowledge made it apparent that the overproduction theory was not capable of explaining many features of the diabetic state and ideas have gradually crystallized into a new concept which involves also the impaired ability of the diabetic organism to utilize carbohydrate." (Best, C.)

The determination of glucose production rates and glucose utilization has been the subject of several studies. These studies, however, have produced contradictory results. In diabetic subjects glucose production rates have been variously reported as decreased (Reichard *et al.* 1961; Kalant *et al.* 1963), normal (Myers, 1950; Wahren *et al.* 1972) and increased (Forbath and Hetenyi, 1966; Bowen and Moorhouse, 1973). Glucose utilization in diabetes has been reported to be decreased (Manougian *et al.* 1964; Whichelow and Butterfield, 1971), normal (Shreeve *et al.* 1956; Reichard *et al.* 1961) and increased (Forbath and Hetenyi, 1966). Glucose production rates and peripheral glucose

metabolism have usually been the subject of separate studies, by different workers, on widely different groups of diabetic subjects or experimental animals. Differences in methodology have also added to the difficulty in comparing and interpreting the data.

The nature of the hepatic and peripheral abnormalities in glucose metabolism in diabetes and their relative importance in the development of the acute diabetic syndrome therefore require further elucidation.

#### The Present Study

The object of the present study is to define the pattern of total glucose metabolism, by measuring both hepatic and peripheral metabolism simultaneously, in an animal model as closely related to man as possible. The aspects of glucose metabolism to be studied are the glucose turnover rate (whole body), the extent of glucose recycling, the size of the glucose pool and glucose space, and glucose utilization in the forearm tissues. The inclusion of other metabolic profiles in the study, such as the investigation of aspects of lactate metabolism similar to glucose metabolism as well as the metabolism of triglyceride, glycerol and free fatty acids in forearm tissues, it is hoped, would add to the understanding of glucose metabolism and its relationship to lactate metabolism and certain parameters of lipid metabolism.

A further important objective of the study is to produce diabetes in the animal model, using the diabetogenic drug streptozotocin. All aspects of glucose, lactate and lipid metabolism studied in the normal animal will then be repeated in the diabetic animal. By reference to the control values established for each animal the effects of the acute diabetic syndrome on peripheral and hepatic metabolism would be ascertained. It is also hoped that by studying peripheral and hepatic glucose metabolism simultaneously in the diabetic animal it may be possible to establish whether these abnormalities develop concurrently or whether the one precedes the other, and to ascertain the relative importance of each in the development of hyperglycaemia.

### Glucose Kinetics:

The liver and kidneys are able to synthesize glucose from non carbohydrate precursors (lactate, glycerol and amino acids) by the process of gluconeogenesis. The kidney becomes an important source of glucose synthesis only after prolonged fasting (4-6 weeks), and furthermore the kidney does not contribute to glucose production in diabetes (Felig and Wahren, 1975). Thus in the present study on 16 to 18 hour fasted normal and diabetic animals the measured glucose production rate represents hepatic glucose production.

The glucose production rate may be determined by arterio-hepatic venous catheterization studies or the injection of labelled glucose. The catheterization technique measures hepatic glucose release directly but its use requires specialized laboratory facilities and considerable technical expertise. Furthermore, the placement of permanent indwelling catheters greatly increases the risk of infection especially after the induction of diabetes. An important advantage of the isotopic technique is that the data obtained can also be used to estimate the glucose pool and space and also glucose recycling. A major disadvantage of isotopic techniques, however, is the assumption that removal of the label implies metabolism of the tracer. The tracer may lose its label in the early reactions of a metabolic sequence and return to the compartment being analysed. Loss of label in such cycles will, however, be measured as utilization of the tracer. The turnover rate of a substrate is defined as its rate of production and rate of utilization in the body. In the steady state, i.e. when the substrate concentration (in blood) is relatively constant, the rates of production and utilization will be equal.

(Steady State) Turnover Rate = Rate of Production = Rate of Utilization.

Labelled glucose (tracer) is metabolised in the same way as non labelled glucose (tracee) and the ratio of the turnover rate of the tracer to the turnover rate of the tracee is equal to the ratio of the mass of the tracer to the mass of the tracee (Stetten *et al.* 1951). Thus,

$$\frac{Rt^*}{Rt} = \frac{M^*}{M}$$

where  $Rt^*$  = turnover rate of tracer  
 $Rt$  = turnover rate of tracee  
 $M^*$  = mass of tracer  
 $M$  = mass of tracee.

The ratio of labelled to non labelled substrate in a sample is (by definition) the specific activity (S.A.) of the sample.

$$\text{Hence } \frac{M^*}{M} = \text{S.A.}$$

Thus by substitution,

$$\frac{Rt^*}{Rt} = \text{S.A.}$$

and by rearrangement of the equation,

$$Rt = \frac{Rt^*}{\text{S.A.}}$$

Thus the turnover rate of a substrate ( $Rt$ ) is calculated by determining the turnover rate of the labelled substrate and its specific activity. Following infusion of the tracer at a constant rate the blood specific activity eventually reaches a relatively constant value (steady state or asymptotic specific activity). At this time the rate of removal of tracer equals the rate of entry of tracer. The turnover rate of the tracer ( $Rt^*$ ) is thus equal to the rate of infusion of the tracer. The asymptotic specific activity (S.A.) is determined by the analysis of blood samples taken at intervals during the period of infusion. The administration of a priming dose (i.e., a bolus injection of tracer prior to its infusion) reduces the time taken for the specific activity-time curve to reach an asymptote.

Glucose space measured by tracer kinetics is the apparent volume in which glucose is distributed and glucose pool is defined as the quantity of glucose present in this space. Glucose pool is estimated from the specific activity data by the method of Steele *et al.* (1956). Glucose



space is then calculated from the relationship below.

$$\text{Glucose Space (litres)} = \frac{\text{Glucose Pool (mmol)}}{\text{Blood glucose concentration (mmol/l)}}$$

The calculation assumes a relatively uniform distribution of glucose in the glucose space.

Until recently [U-<sup>14</sup>C] D-glucose was the most widely used tracer for the determination of glucose turnover rates. The metabolism of glucose labelled with <sup>14</sup>C results in the formation of <sup>14</sup>C labelled products especially lactate. The recycling of lactate (Cori cycle) and its utilization in glucose synthesis results in the formation and release of labelled glucose by the liver. This results in a higher asymptotic blood glucose specific activity and therefore underestimation of the turnover rate. Such recycling of the label can also occur via liver glycogen, i.e. labelled glucose → glycogenesis → labelled glycogen → glycogenolysis → labelled glucose. However, in the fasting state glycogenolysis is predominant and recycling by this pathway is probably relatively insignificant. When glucose is labelled with tritium recycling of the label does not occur and thus the turnover rate is more accurately estimated. Katz and Dunn (1967) reported the use of [2-<sup>3</sup>H] D-glucose as a tracer for measuring glucose turnover rate. This isotope loses the tritium in the hexose isomerase reaction of glycolysis (glucose 6-phosphate  $\rightleftharpoons$  fructose 6-phosphate) as tritiated water. The high turnover rate of the hexose phosphate pool results in removal of most of the tritium from glucose and hence extensive recycling of the label via compounds such as lactate does not occur. The simultaneous use of [U-<sup>14</sup>C] D-glucose and [2-<sup>3</sup>H] D-glucose permits the estimation of glucose recycling (Katz *et al.* 1974a). Recycling provides an index of glucose production from lactate (Cori cycle) and the value of the glucose turnover rate after subtraction of recycling would be an approximate estimate of glucose produced from non lactate sources (Kreisberg, 1972).

### Lactate Kinetics:

The determination of lactate turnover rates, lactate pool and lactate space is based on similar principles to glucose kinetics. The lactate tracer used in this study is [U- $^{14}\text{C}$ ] L-lactate. Lactate labelled with  $^{14}\text{C}$  is subject to recycling in the same way as glucose and thus the turnover rate could in theory be underestimated. However, Kreisberg (1972) has suggested that tracer methods may possibly overestimate lactate production rates. Exchange of label between lactate and the pyruvate-alanine system would result in overestimation of the lactate turnover rates. Unfortunately, other isotopes of lactate suitable for estimation of turnover rates are not commercially available at the time of this study and, despite its limitations, the use of [U- $^{14}\text{C}$ ]L-lactate will provide information on the pattern of lactate metabolism in the normal animal and outline disturbances in diabetes.

### Forearm Metabolism:

The use of the forearm technique to study the peripheral metabolism of substrates is well documented (Butterfield and Holling, 1959; Andres *et al.* 1962; Christensen and Orskov, 1968; Ganda *et al.* 1971). The technique involves simultaneous sampling of arterial and venous blood to determine substrate concentrations and the estimation of blood flow to the forearm.

Venous blood was obtained (without stasis) from the deep antecubital vein of the forearm being studied. Arterial blood was obtained from any convenient site usually the tibial artery because of its superficial position in the baboon. Forearm blood flow was determined by strain gauge plethysmography. The principles and use of this technique are well documented (Whitney, 1953; Greenfield *et al.* 1963; Pauca and Sykes, 1967).

The forearm technique is ideal for the study of peripheral metabolism. It allows an assessment of peripheral metabolic exchange in a localized

region of the body without divorcing it from the influence and control of the rest of the body.

The Experimental Animal:

The proposed investigation of glucose and lactate metabolism involves repeated injections of radioactive compounds over a short period of time. In addition, the fact that peripheral glucose metabolism would be studied simultaneously made experiments on human subjects unethical. Thus the experiments will be performed on an animal model as close as possible to man. This has a particular advantage in a study of glucose and lactate metabolism because control values can be established for each individual animal and serve as a reference for the quantitative assessment of metabolic abnormalities occurring after the production of diabetes. In the present study the baboon (*Papio ursinus*) was used as the experimental animal. As a primate, it is closely related to man taxonomically. Thus, in comparison to other animals, the metabolic profile in the normal baboon, and hence the metabolic abnormalities resulting from diabetes, are more likely to resemble that in man. Furthermore, there are few reports of glucose and lactate metabolic studies in primates and, to the author's knowledge, none in the baboon.

SUMMARY.

Hepatic and peripheral glucose metabolism will be studied simultaneously in the intact normal baboon. Other metabolic parameters such as lactate kinetics and aspects of forearm lipid metabolism will also be investigated. The data obtained in the normal baboon would provide reference values with which to compare the results of experiments performed after the production of an acute diabetic syndrome with streptozotocin.

From these metabolic studies it is hoped that it will be possible to

define the nature and magnitude of the metabolic differences between the normal and diabetic state, the relative importance of peripheral and hepatic abnormalities in the development of the acute diabetic syndrome, and any interrelationships between the various aspects of glucose, lactate and lipid metabolism studied.

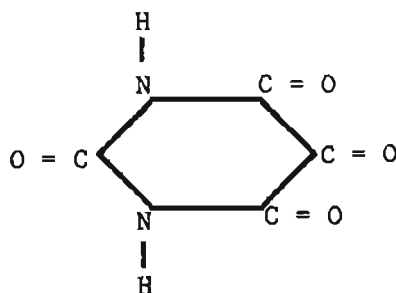
## CHAPTER TWO

## DRUG INDUCED DIABETES IN ANIMALS

A heterogeneous group of compounds with widely different chemical structures have been found to have a relatively selective cytotoxic effect on pancreatic  $\beta$  cells. The destruction of  $\beta$  cells results in an acute insulin deficiency which precipitates diabetes mellitus. These diabetogenic compounds include substances such as dehydroascorbic acid, styrylquinoline, diphenylthiocarbazon, alloxan and streptozotocin. Of these alloxan has for many decades been the most widely used and studied diabetogenic drug. However, in recent years it has been surpassed in usage by streptozotocin, and the already extensive literature on Streptozotocin Diabetes is continually increasing. A brief review of alloxan and streptozotocin experimental diabetes follows.

## ALLOXAN

The synthesis and chemical nature of alloxan was first described in 1838 by Wohler and Liebig. The structure of alloxan is shown below.



Alloxan

Dunn *et al.* (1943) while studying the effects of alloxan on the rabbit kidney observed fortuitously that its injection produced partial or total necrosis of the pancreatic islets. In the same year alloxan was shown to produce a diabetic state in the rat (Dunn and McLetchie, 1943), in the rabbit (Bailey and Bailey, 1943; Brunschwig *et al.* 1943) and in the dog (Goldner and Gomori, 1943). The features of alloxan diabetes were identical to those of human diabetes - hyperglycaemia, glycosuria, polydipsia, polyuria, loss of body weight, hyperlipidaemia, ketonuria and acidosis. After receiving an injection of alloxan, non-fasted animals showed a triphasic blood glucose pattern (Brunschwig *et al.* 1943).

Phase 1 - early hyperglycaemia of short duration (1-4 hours)

Phase 2 - hypoglycaemia which may last up to 48 hours.

Phase 3 - chronic hyperglycaemia of long duration.

In fasted animals the early hyperglycaemia was attenuated or lacking and the hypoglycaemia was more severe (Wrenshall *et al.* 1950). This suggested that glycogenolysis contributed to the initial hyperglycaemia and prevented the development of severe hypoglycaemia subsequently. A decrease in plasma insulin to very low levels coincided with the early hyperglycaemic phase (the cause of the decrease is not known). Insulin levels then increased above normal, being highest at the time of most intense hypoglycaemia (probably the result of leakage from damaged  $\beta$  cells). After the establishment of permanent alloxan diabetes plasma insulin levels were very low or unmeasurable (Lundquist and Rerup, 1967; Morgan and Lazarow, 1963).

The development of alloxan diabetes can be attenuated or inhibited by:

(1) Clamping off the pancreatic blood supply from the general circulation for five minutes after an injection of alloxan. This indicates a short half-life of alloxan in blood (Gomori and Goldner, 1945).

(2) The injection of reducing agents such as sodium bisulfite or 3,4-diaminotoluene before alloxan (Weinglass *et al.* 1945) or by increasing the level *in vivo* of physiological compounds acting as reducing agents, prior to alloxan administration, e.g. glutathione and cysteine (Lazarow, 1947).

(3) Injection of certain metallic ions such as zinc, cobalt and iron (Lazarow and Patterson, 1951) prior to alloxan administration.

(4) Injection of compounds such as nicotinic acid and nicotinamide (Lazarow *et al.* 1950) and monoamine oxidase inhibitors (Buschiazzo *et al.* 1967) prior to alloxan administration.

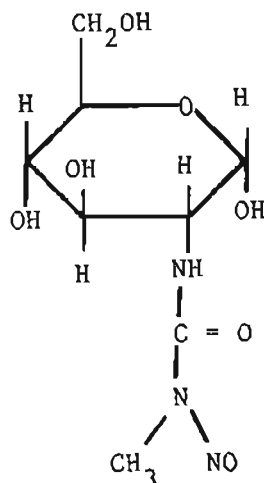
The duration of alloxan diabetes is related to severity. High doses which cause an immediate and nearly total destruction of  $\beta$  cells result in a severe diabetes with death occurring within a few weeks. A dose causing sub-total destruction of the  $\beta$  cell population results in different effects in different species. In dogs and cats a permanent diabetes with ketoacidosis develops and insulin therapy is required for survival. In rodents the diabetes may be permanent or spontaneous remission may occur (spontaneous recovery is particularly evident in rats and mice). The recovery from diabetes is thought to be the consequence of either a multiplication of surviving  $\beta$  cells or the formation of new  $\beta$  cells from the duct epithelium of the exocrine pancreas (Bunnag *et al.* 1967). If more than the diabetogenic dose is given the organ most frequently reported to be seriously affected is the kidney (Rerup, 1970). The renal lesions may be mild (vacuolation, hydropic changes and desquamation of tubular cells) or severe, causing renal failure from tubular necrosis. Liver damage (fatty infiltration and liver necrosis) has also been reported (Houssay *et al.* 1946; Lazarow and Palay, 1946).

The mechanism of action of alloxan on the islet  $\beta$  cell is not precisely known. The site of action is probably the  $\beta$  cell membrane. It has a short half-life in blood and binding to the  $\beta$  cell membrane is completed within a few minutes. The histological and most of the biochemical changes which occur later than five minutes after the injection are secondary changes to the initial effect on the  $\beta$  cell and are not due to a continuing direct alloxan effect.

#### STREPTOZOTOCIN

Streptozotocin, a broad spectrum antibiotic produced by *streptomyces*

*achromogenes* (Vavra *et al.* 1959-60) is a remarkable compound. Its isolation and characterization was reported by Herr *et al.* (1959-60). An unexpected property of streptozotocin was its highly specific diabetogenic action, first reported by Rakieta *et al.* (1963). Besides being an antibiotic and a diabetogenic agent it has also been reported to have both tumorigenic (Arison and Feudale, 1967; Rakieta *et al.* 1976b) and antitumour effects (Broder and Carter, 1973; Schein *et al.* 1973a). Streptozotocin consists of 1-methyl-1-nitrosourea linked to carbon 2 of 2-deoxy D-glucose (Herr *et al.* 1967) and its structural formula is shown below.



Streptozotocin

Diabetogenesis: In the original study (Rakieta *et al.* 1963) it was reported that streptozotocin produced a sustained and prolonged hyperglycaemia in dogs and rats. The diabetic animals showed polyuria, polydipsia and glycosuria but no ketonuria and their growth and food consumption was not significantly different from controls. On the basis of histological and biochemical evidence, Rakieta *et al.* (1963) concluded that Streptozotocin Diabetes was caused by degeneration of the islets of Langerhans associated with a marked decrease or complete absence of granules in the  $\beta$  cells. In a later study Arison *et al.* (1967) reported that rats showed total .



degranulation of  $\beta$  cells without cytologic disintegration, 48 hours after a single intravenous dose of streptozotocin, 65 mg/kg. In the same year (1967) Junod *et al.* reported that seven hours after a single intravenous dose of streptozotocin, 65 mg/kg, rats showed massive necrosis of the  $\beta$  cells with cellular disintegration and often nearly complete karyolysis. Remaining  $\beta$  cells were rare and if present were usually degranulated. The  $\alpha$  cells and exocrine tissue remained intact without any evidence of damage. There was some evidence of focal hepatic necrosis after 2-36 hours but no evidence of necrosis in the kidney or other tissues.

Both Arison *et al.* (1967) and Junod *et al.* (1967) injected the same dose of streptozotocin into rats and yet markedly different effects on the  $\beta$  cells were observed. Two reasons are suggested to account for these results. Firstly, in earlier studies, including that of Arison *et al.* (1967) there was considerable doubt about the purity of the streptozotocin used. Junod *et al.* (1967) took special care to ensure that the streptozotocin they used conformed to strict standards of purity and that contaminants were eliminated. Secondly, streptozotocin if dissolved in saline or distilled water at neutral pH decomposes rapidly with the evolution of gas. Its stability in solution is optimal at pH 4 and low temperature (Rakieten *et al.*, 1963). In the study by Junod *et al.* (1967) streptozotocin was dissolved in saline and citric acid added immediately to adjust the pH of the solution to 4.5. Arison *et al.* (1967) dissolved streptozotocin in saline and in their report it is not mentioned if the pH of the solution was adjusted between 4 and 5. Decomposition of the drug, proportional to the length of the interval between preparation and injection, was therefore a possible factor.

In a later study Junod *et al.* (1969) investigated the relationship between the dose of streptozotocin injected and the metabolic response in rats. A dose of 25 mg/kg produced slight transient glycosuria and a small but significant decrease in pancreatic immunoreactive-insulin, with no other gross abnormality. A dose of 35 mg/kg produced a definite but mild hyperglycaemia after 24 hours. At this dose spontaneous recovery was noted in 25% of the animals. A dose of 65 mg/kg produced permanent hyperglycaemia after 24 hours, with a 50% fall in pancreatic immunoreactive-insulin and low serum immunoreactive-insulin. There was

no ketonuria at any time thereafter. A dose of 100 mg/kg produced a more pronounced decrease of pancreatic immunoreactive-insulin with an acute and progressive diabetes with ketonuria in the first 24 hours and death in 2-3 days. This study showed clearly that the severity of Streptozotocin Diabetes was dose related and that there was a wide margin between diabetogenic and toxic doses.

The diabetogenic action of streptozotocin has been demonstrated in many other species, including the hamster (Little, 1966), mouse, guinea pig (Brosky and Logothetopoulos, 1968), and monkey (Pitkin and Reynolds, 1970).

Mechanism of Action: Initially it was thought that streptozotocin probably had a similar mechanism of action to alloxan. Similarities exist in the pathophysiology of alloxan and streptozotocin induced diabetes. Both are specific in their  $\beta$  cell toxicity. Streptozotocin, like alloxan, produces a triphasic blood glucose response - initial hyperglycaemia corresponding with depletion of liver glycogen (peak at 3 hours), hypoglycaemia with the lowest blood sugar levels at 7-9 hours and corresponding with elevated plasma immunoreactive-insulin levels, and thereafter (after 24 hours) a chronic hyperglycaemia (Schein *et al.* 1967; Junod *et al.* 1967; Schein and Bates, 1968). Another similarity with alloxan is that the injection of nicotinamide (500 mg/kg) prior to streptozotocin protects against diabetogenesis in mice and rats (Schein *et al.* 1967; Junod *et al.* 1969).

There are, however, many differences between alloxan and streptozotocin diabetogenesis. One of these is that a wide range of chemicals (e.g. cysteine, glutathione, metallic ions and nicotinic acid) inhibit the diabetogenic action of alloxan but not that of streptozotocin. Of major significance is that the injection of streptozotocin was shown to result in a depression of oxidised and reduced nicotinamide adenine dinucleotide (NAD) concentrations in mouse liver (Schein *et al.* 1967; Schein and Loftus, 1968). The depression of NAD levels was dose related.

These studies also demonstrated that if nicotinamide (500 mg/kg) was injected prior to streptozotocin the depression of NAD levels in the liver was prevented, despite the uptake of streptozotocin by the liver being unaffected. Although nicotinamide protects against alloxan diabetogenesis, the injection of alloxan does not produce any significant depression of NAD levels. These studies suggested that the diabetogenic action of streptozotocin was probably due to inhibition of NAD biosynthesis in pancreatic islet cells.

Further studies (Schein *et al.* 1973b) supported this hypothesis. The injection of streptozotocin (200 mg/kg) produced a significant decrease in NAD concentration of individual isolated mouse pancreatic islets, from a control mean value of 0,78 to 0,11 pmol after 2 hours. The decrease in NAD concentration was prevented if the animals were given an intraperitoneal injection of nicotinamide (500 mg/kg) ten minutes prior to streptozotocin injection. The incorporation of labelled nicotinamide into NAD by pancreatic islets was also measured and was found to be reduced by 83% in streptozotocin-treated animals when compared to controls. On the basis of their studies Schein *et al.* (1973b) proposed that the diabetogenic action of streptozotocin was related to a decrease in  $\beta$  cell NAD concentration resulting from decreased tissue uptake of precursors and decreased synthesis of NAD. It would also appear that  $\beta$  cells were relatively more sensitive to low levels of NAD than other cells, such as hepatic cells.

Studies on mice confirmed that streptozotocin produced a marked reduction in islet cell NAD concentration (Gunnarson *et al.* 1974; Anderson *et al.* 1974). Gunnarson *et al.* (1974) also showed that the  $\beta$  cell toxicity of streptozotocin was greater than that of 1-methyl-1-nitrosourea, the aglycone of streptozotocin. Anderson *et al.* (1974) found that streptozotocin (200 mg/kg) reduced mean islet NAD concentration from 0,78 to 0,15 pmol/islet and was diabetogenic while 1-methyl-1-nitrosourea (100 mg/kg) reduced mean islet NAD concentration to 0,58 pmol/islet and was not diabetogenic. On a molar basis the dose of 1-methyl-1-nitrosourea used was 1,3 times the dose of streptozotocin. (Diabetogenic doses of alloxan did not decrease islet NAD concentration.) Anderson *et al.* (1974)

also measured the in vitro uptake of methyl-<sup>14</sup>C streptozotocin by islet cells and found it to be 3,8 times greater than that of methyl-<sup>14</sup>C 1-methyl-1-nitrosourea.

These studies suggest that 1-methyl-1-nitrosourea is the cytotoxic moiety of streptozotocin and that the glucose portion of streptozotocin facilitates its uptake into the  $\beta$  cell. Inside the  $\beta$  cell 1-methyl-1-nitrosourea causes a marked depression of NAD concentration, probably by inhibiting its synthesis from nicotinamide. Direct evidence to support the hypothesis that streptozotocin inhibits NAD biosynthesis from nicotinamide is lacking. The activity of enzymes involved in the synthesis and degradation of NAD have been reported as unaltered after the administration of 1-methyl-1-nitrosourea (Schein, 1969), while streptozotocin induced alterations in the activity of these enzymes in islet cells has not been reported. Furthermore, streptozotocin is metabolized rapidly in the liver and has a short half-life in blood (Schein *et al.*, 1973a; Karunanayake *et al.*, 1976) and produces maximal islet cell NAD depression two hours after injection (Schein *et al.*, 1973b). The injection of nicotinamide even two hours after streptozotocin has been shown to have a protective effect against diabetogenesis (Dulin and Wyse, 1969). This suggests NAD biosynthesis from nicotinamide, implying unimpaired synthetic pathways even two hours after streptozotocin.

The possibility that streptozotocin might cause depletion of NAD levels by transmethylation of nicotinamide to N-methyl-nicotinamide, thereby making it unavailable for NAD biosynthesis, was recently investigated (Karunanayake *et al.*, 1976). The results show that nicotinamide was not methylated by a transfer of the free methyl group of streptozotocin.

Thus despite extensive investigation, the mechanism of action of streptozotocin is still not completely understood.

#### CHOICE OF DIABETOGENIC DRUG.

Alloxan and streptozotocin are the two most widely used diabetogenic drugs.

Several studies in which both agents have been used indicate that streptozotocin has higher specificity and lower toxicity than alloxan.

Rats made diabetic with alloxan (65 mg/kg) showed hyperglycaemia, striking elevation of plasma free fatty acids and blood ketones, and abnormal concentrations of cardiac glycolytic intermediates and glycogen (Mansford and Opie, 1968). On the other hand, rats made diabetic with streptozotocin (65 mg/kg) showed hyperglycaemia without any significant abnormality in the concentration of free fatty acids, ketones or cardiac glycolytic intermediates and glycogen. In another study (Hoftiezer and Carpenter, 1973) rats given equimolar doses of alloxan and streptozotocin showed a mortality ten times greater with alloxan, a consistently lower level of hyperglycaemia with streptozotocin, and the streptozotocin treated rats maintained their weight while alloxan treated rats lost weight. In a study on vervet monkeys, in which alloxan (50 mg/kg) or streptozotocin (60 mg/kg) was injected to produce diabetes, considerable variation in response was observed with alloxan while streptozotocin produced a limited range of response and its effects were therefore more reproducible (White *et al.* 1974).

The results of these studies indicate that streptozotocin is the drug of choice for producing diabetes in animals.

#### SUMMARY

- (1) Streptozotocin consists of a 1-methyl-1-nitrosourea moiety, which is probably the cytotoxic moiety, and this is linked to carbon 2 of 2-deoxyglucose which facilitates its cellular uptake or interaction with the  $\beta$  cell membrane.
- (2) Streptozotocin is subjected to rapid metabolic degradation in the liver and its cytotoxic action is complete soon after its injection.
- (3) The specificity of streptozotocin for the islet  $\beta$  cell is the result of greater sensitivity of the  $\beta$  cell to the NAD depletion caused

by streptozotocin rather than to its greater uptake by the  $\beta$  cell.

(4) The damage to islet  $\beta$  cells is the result of a streptozotocin induced depression of cellular NAD concentration below a critical level. The mechanism by which streptozotocin depresses cellular NAD levels is not known.

(5) The pathogenesis of  $\beta$  cell destruction by alloxan and streptozotocin differ in mechanism, despite a common end result.

(6) Streptozotocin appears to have a greater specificity for the islet  $\beta$  cell and lower overall toxicity than alloxan.

## CHAPTER THREE

## MATERIALS - METHODS - TECHNIQUES

ANIMALS: All experiments were carried out on male, adult baboons (*Papio ursinus*). The ten baboons used for metabolic studies varied in weight from 23 to 32 kilograms. The animals were housed individually in adjoining wire mesh cages measuring 2,5 x 1,5 x 1,0 metres, each having a shelter and access to water. The animals were fed on a diet of cereals, fruit and vegetables for several months prior to being studied. All experiments were carried out in the postabsorptive state, the animals being fasted for a period of 16-18 hours.

One of the cages was modified so that it was possible to force the animal against the back of the cage and thus immobilise it. Anaesthetic injections could then be administered in complete safety. The baboons were studied individually and each animal was transferred from its usual cage to the modified cage, and it would remain there till all experiments were completed, and then returned to its original cage.

SEDATION: Sernylan (Phencyclidine Hydrochloride : Parke-Davis) and Sagatal (Pentobarbitone Sodium : May and Baker) were used to produce sedation and relaxation.

Sernylan has been extensively used in primates and it has been shown to have a wide margin of safety with minimal side effects (Vondruska, 1965; Mortelmans, 1969; Kalter, 1969). In baboons a dose of 3 mg/kg intramuscularly produces a calming effect and then prostration in five to ten minutes. This is followed by sedation of four to six hours duration and it is sufficiently deep to allow injections, venepuncture or catheterisation in complete safety.

During preliminary experiments it was noticed that Sernylan produced an increase in muscle tone. This made it difficult to maintain the forearm in a relaxed and extended position and thus proper application of

the strain gauge (for forearm blood flow determination) was difficult. Furthermore minor fluctuations in forearm muscle tone tended to occur and this disturbed the sensitive strain gauge and interfered with flow recordings. The administration of Sagatal in low doses, in addition to Sernylan in a reduced dose, produced adequate sedation and prevented an increase in muscle tone occurring.

Thus in all baboons in this study, both before and after diabetes, the standard regime used for sedation was intramuscular injections of Sernylan 2 mg/kg, and Sagatal 8 mg/kg. (Usual doses for anaesthesia are 7-8 mg/kg intramuscularly for Sernylan and 30 mg/kg intravenously for Sagatal.) If further sedation was necessary during the experiment then Sernylan alone in a dose of 1 mg/kg, intramuscularly, was adequate.

GLUCOSE KINETICS: Glucose Turnover Rates, Glucose Recycling, Glucose Pool and Glucose Space were determined.

(1) Experimental Procedure: Seventy five microcuries ( $\mu\text{Ci}$ ) [ $2\text{-}^3\text{H}$ ] D-glucose and fifty  $\mu\text{Ci}$  [ $\text{U-}^{14}\text{C}$ ] D-glucose (Amersham : England) were mixed well in 10 ml of sterile isotonic saline. From this mixture a 6,4 ml aliquot containing 48  $\mu\text{Ci}$  of [ $2\text{-}^3\text{H}$ ] D-glucose and 32  $\mu\text{Ci}$  of [ $\text{U-}^{14}\text{C}$ ] D-glucose was added to 340 ml sterile isotonic saline and thoroughly mixed. The remaining 3,6 ml containing 27  $\mu\text{Ci}$  [ $2\text{-}^3\text{H}$ ] D-glucose and 18  $\mu\text{Ci}$  of [ $\text{U-}^{14}\text{C}$ ] D-glucose was injected intravenously as a priming dose. The saline, containing the  $^3\text{H}$  and  $^{14}\text{C}$  labelled glucose, was then infused through a venous catheter at a constant rate of 1,62 ml per minute by an infusion pump (Harvard Apparatus, Model 1201) so that the ratio of priming dose ( $\mu\text{Ci}$ ) to infusion rate ( $\mu\text{Ci/hr}$ ) was 2:1. At this ratio the blood glucose specific activity-time curve approaches an asymptote after 60 to 90 minutes (Kreisberg *et al.* 1970), and the errors incurred in the measurement of pool size (by assuming instantaneous mixing in the blood glucose pool) are reduced (Steele *et al.* 1956).

The infusion was continued for three hours and during this time 11 ml of venous blood were withdrawn from the femoral vein at 45, 90, 105, 120, 135, 150 and 180 minutes after commencing the infusion. Frequency of sampling



was increased between 90 and 150 minutes as the specific activity-time curve was very likely to have reached the asymptotic value during this period. Immediately after blood samples were obtained, a 0,1 ml aliquot was pipetted and deproteinized with 1,0 ml of 0,33 M cold perchloric acid (4°C) and a 10 ml aliquot was deproteinized with an equal volume of 0,66 M cold perchloric acid. After three hours the infusion was stopped and an aliquot of the mixture being infused was taken and stored at -4°C for subsequent glucose  $^{14}\text{C}$  and  $^3\text{H}$  counting.

(2) Analytical Procedures: The deproteinized blood samples were centrifuged. The supernatant obtained from the mixture of 0,1 ml blood and 1,0 ml perchloric acid was used to determine blood glucose concentration by the hexokinase : glucose-6-phosphate-dehydrogenase method (Appendix One page 121). The supernatant from the mixture of 10 ml blood and 10 ml perchloric acid was filtered, and 1,0 ml of the filtrate was used for determining blood lactate concentration by the lactate dehydrogenase method (Appendix Two page 123). The rest of the filtrate was neutralized (pH 7,0 to 7,4) with potassium hydroxide and kept at 4°C for 30 minutes, to allow complete settling of the resulting potassium perchlorate. The clear supernatant was chromatographed onto prepared columns containing Biorad AG-1x8, 200-400 mesh, chloride free resin (Appendix Three page 125). Glucose was eluted by washing the column with 20 ml distilled-deionised water. A 0,2 ml aliquot was used to determine the concentration of glucose in the eluate (Appendix One page 121). A 4 ml aliquot of the eluate was evaporated to dryness in a vacuum at 70°C in order to eliminate any tritiated water present in the plasma. The residue was dissolved in 1,0 ml distilled-deionised water and counted in 10 ml Bray's Solution (New England Nuclear : Massachusetts). The  $^{14}\text{C}$ -glucose and  $^3\text{H}$ -glucose counts per minute (cpm) were determined simultaneously in a liquid scintillation counter (Packard Tri-Carb Liquid Scintillation Spectrometer Model 544) using two channels. The cpm was corrected to dpm (disintegrations per minute) by reference to quenched standard curves which indicate the counting efficiencies of  $^{14}\text{C}$  and  $^3\text{H}$  in their respective channels and the overlap of  $^{14}\text{C}$  counts into the  $^3\text{H}$  channel. A brief description of the preparation and application of quenched standard curves in the simultaneous counting of double labelled samples is presented in Appendix Four, page 126.

(3) Calculations: Glucose turnover rates were calculated from the mean blood glucose specific activity at the asymptote, using the relationship described in Chapter One.

$${}^{14}\text{C} \text{ or } {}^3\text{H} \text{ Turnover rate} \quad = \quad \frac{{}^{14}\text{C} \text{ or } {}^3\text{H} \text{ glucose infusion rate (dpm/min)}}{{}^{14}\text{C} \text{ or } {}^3\text{H} \text{ blood glucose specific activity (dpm}/\mu\text{mol)}}$$

Turnover rates were then expressed as  $\mu\text{mol/kg/hr}$ .

In contrast to  $[\text{U-}^{14}\text{C}]$  D-glucose, the use of  $[\text{2-}^3\text{H}]$  D-glucose as a tracer is not associated with extensive recycling of the label. Thus when both tracers are used simultaneously the amount of glucose recycled is defined by the relationship below (Katz *et al.* 1974a).

$$\text{Glucose Recycling } (\mu\text{mol/kg/hr}) = \frac{[\text{2-}^3\text{H}] \text{ glucose turnover rate}}{[\text{U-}^{14}\text{C}] \text{ glucose turnover rate}}$$

Glucose pool (mmol) was calculated from the specific activity curve using the formula of Steele *et al.* (1956). Glucose space was calculated by the relationship below.

$$\text{Glucose Space (litres)} = \frac{\text{Glucose Pool (mmol)}}{\text{Mean blood glucose concentration (mmol/l)}}$$

Glucose pool and glucose space were also expressed relative to body weight (the pool as  $\mu\text{mol/kg}$  and the space as a percentage of body weight).

LACTATE KINETICS: The turnover rate of lactate, lactate pool and lactate space were determined.

(1) Experimental Procedure: Fifty  $\mu\text{Ci}$  of  $[\text{U-}^{14}\text{C}]$  L-lactate (Amersham : England) were mixed in 10 ml sterile, isotonic saline. From this mixture

an aliquot of 8,7 ml containing 43,5  $\mu\text{Ci}$  [ $\text{U-}^{14}\text{C}$ ] L-lactate was added to 316 ml sterile isotonic saline and thoroughly mixed. The remaining 1,3 ml containing 6,5  $\mu\text{Ci}$  [ $\text{U-}^{14}\text{C}$ ] L-lactate was injected intravenously as a priming dose. The saline containing the  $^{14}\text{C}$  labelled lactate was then infused through a venous catheter by an infusion pump (Harvard Apparatus, Model 1201) at a constant rate of 1,62 ml per minute, so that the ratio of priming dose ( $\mu\text{Ci}$ ) to infusion rate ( $\mu\text{Ci/hr}$ ) was 1:2. At this ratio blood lactate specific activities approach an asymptotic value after about 120 minutes (Kreisberg *et al.* 1970). The infusion was continued for three hours, and 11 ml of venous blood were withdrawn from the femoral vein at 45, 90, 105, 120, 135, 150 and 180 minutes after commencing the infusion. Deproteinization of 0,1 ml blood (for glucose determination) and 10 ml blood (for lactate specific activity determination) with perchloric acid were carried out as described in the experimental procedure for glucose kinetics. At the end of the infusion period an aliquot of the remaining saline was taken and stored at  $-4^{\circ}\text{C}$  for subsequent lactate  $^{14}\text{C}$  counting.

(2) Analytical Procedures: Following deproteinization the samples were processed (centrifuged, filtered, neutralized and an aliquot for blood lactate assay taken) as described in analytical procedures for glucose kinetics. The clear supernatant was chromatographed on Biorad AG-1x8, 200-400 mesh, chloride free resin (Appendix Three page 125) and glucose was removed by washing with 20 ml distilled, deionised water. The column was then washed with 25 ml 0,05 M formic acid, the effluent being discarded. Lactate was then eluted with 25 ml 0,2 M formic acid. The pH of the lactate eluate was adjusted to between 7,0 and 7,4 with potassium hydroxide. A 0,6 ml aliquot of the eluate was used to determine the concentration of lactate by the lactate dehydrogenase method (Appendix Two page 123). A 4 ml aliquot was counted in 15 ml Bray's Solution (New England Nuclear : Massachussets) in a Packard liquid scintillation counter using the  $^{14}\text{C}$  channel only. The lactate  $^{14}\text{C}$  cpm were corrected to dpm by reference to a standard quench curve (Appendix Four).

(3) Calculations: Lactate turnover rate was calculated from the mean blood lactate specific activity at the asymptote, using the relationship described in Chapter One.

$$\text{Lactate turnover rate} = \frac{{}^{14}\text{C lactate infused (dpm/min)}}{\text{Blood lactate specific activity (dpm/\mu\text{mol})}}$$

$\mu\text{mol/min}$

Lactate turnover rates were then expressed as  $\mu\text{mol/kg/hr}$ .

Lactate pool (mmol) was calculated by using the method as for glucose (Steele *et al.* 1956). Minor modifications in the calculations were necessary in order to take into account the different molecular weight of lactate.

Lactate space was calculated by the relationship below.

$$\text{Lactate space (litres)} = \frac{\text{Lactate pool (mmol)}}{\text{Mean blood lactate concentration (mmol/litre)}}$$

Lactate pool and space were also expressed relative to body weight.

FOREARM METABOLISM: The nett metabolism (uptake or release) in the forearm of glucose, lactate, triglyceride, free fatty acid and glycerol was determined by the forearm technique. This involved the simultaneous measurement of forearm blood flow and the sampling of arterial and venous blood to estimate metabolite concentrations.

Arterial blood was obtained from the tibial artery, which is superficial for most of its course in the leg. Venous blood was obtained from a deep antecubital vein of the forearm being studied. A 0,1 ml aliquot of arterial and venous blood was deproteinized with 1,0 ml 0,33 M perchloric acid, centrifuged, and the supernatant used for determining glucose concentration by the hexokinase : glucose-6-phosphate dehydrogenase method (Appendix One page 121). A 0,5 ml aliquot of arterial and venous blood was deproteinized with 1,0 ml 0,66 M perchloric acid, centrifuged, and the supernatant used for determining lactate concentration by the lactate dehydrogenase method (Appendix Two page 123). The remaining arterial and venous blood samples (about 5 ml of each) were

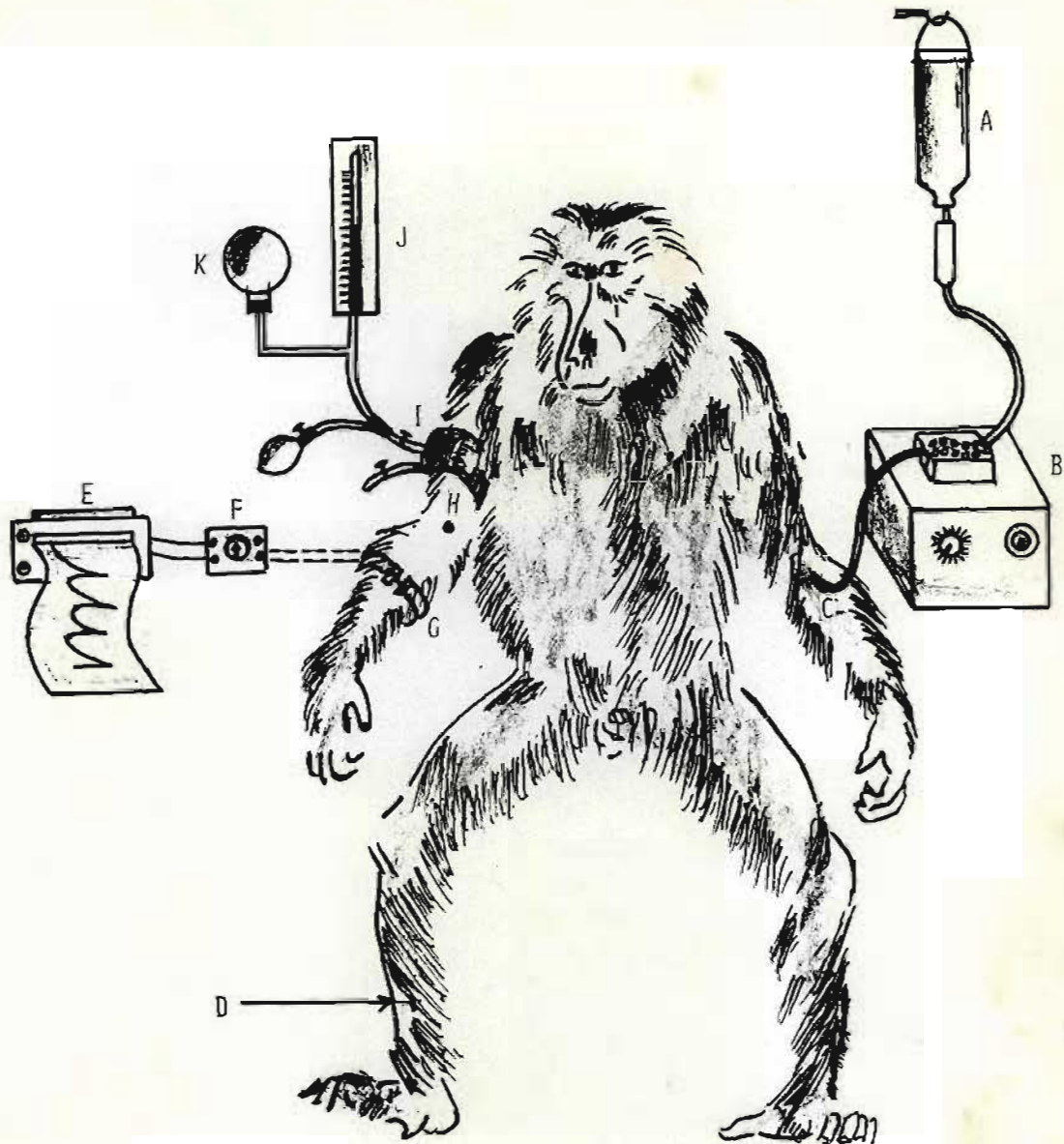
centrifuged and the arterial and venous plasma obtained were used for the determination of triglyceride, free fatty acid and glycerol concentrations by the methods described in Appendices Five, Six and Seven respectively (pp. 131-136).

Forearm blood flow was measured by strain gauge plethysmography. The hand was included in flow measurements because, in the baboon, the hand is small and consists of bones, tendons and skin with virtually no muscle or adipose tissue, and the blood supply to the hand is insignificant relative to the total forearm blood supply. A double stranded strain gauge (Whitney, 1953) was applied around the forearm, about 6 to 8 centimetres below the elbow. The gauge was connected to a plethysmograph (Parkes, Model 270) and a recorder (Unicorder, 4225m). The gauge circumference (and hence tension) was adjusted so that a satisfactory baseline was recorded. A sphygmomanometer cuff was applied above the elbow. The cuff was connected by a piece of rubber tubing to a glass reservoir which was connected to a mercury manometer (Figure 3.1). The connecting tube between the reservoir and the cuff was clamped and the pressure in the reservoir raised to 130 mm Hg. After a few seconds the clamp on the tubing connecting the reservoir to the cuff was removed. This results in immediate inflation of the cuff to a pressure of 60 mm Hg. (The level to which the pressure in the glass reservoir must be raised in order to produce a pressure of 60 mm Hg in the cuff was determined previously, by trial and error, to be 130 mm Hg.) At this pressure arterial flow to the forearm continues but venous outflow is prevented. The increase in forearm circumference in the first few seconds is directly proportional to the arterial blood flow, and is detected by the sensitive strain gauge and recorded. After each recording calibration was carried out with the gauge in position on the forearm by altering the circumference of the gauge by a known amount. The structure and use of the gauge, including a theoretical validation of the method has been described by Whitney (1953).

Forearm metabolism of a metabolite was calculated as the product of the arterio-venous difference (mean of duplicate analyses) and the forearm blood flow (mean of four estimations made in one minute). For the calculation of forearm metabolism of triglyceride, free glycerol and free fatty acid (concentrations determined in plasma), the haematocrit

reading was used to correct forearm blood flow to plasma flow.

The general layout during a typical experiment to determine glucose or lactate kinetics and forearm metabolism is illustrated in Figure 3.1.



- |   |  |   |                      |
|---|--|---|----------------------|
| A | SALINE WITH LABELLED GLUCOSE<br>OR LACTATE | F | PLETHYSMOGRAPH       |
| B | CONSTANT RATE INFUSION PUMP                | G | STRAIN GAUGE         |
| C | INFUSION SITE                              | H | VENOUS SAMPLING SITE |
| D | ARTERIAL SAMPLING SITE                     | I | BLOOD PRESSURE CUFF  |
| E | RECORDER                                   | J | SPHYGMOMANOMETER     |
|   |  | K | GLASS RESERVOIR      |

FIGURE 3.1 : EXPERIMENTAL LAYOUT.

## CHAPTER FOUR

## STREPTOZOTOCIN DIABETES IN THE BABOON

While there is extensive documentation of the use of both alloxan and streptozotocin in the production of experimental diabetes in animals such as the rat and mouse, there have been only a few such studies on primates (White *et al.* 1974; Pitkin and Reynolds, 1970). To the author's knowledge the diabetogenic effect of streptozotocin has never been investigated in the baboon. The baboon (*Papio ursinus*) is found in rocky or mountainous regions near forested areas and because of its ready availability in Southern Africa it has been used widely as a research animal.

In the present study the diabetogenic action of streptozotocin was investigated in the baboon. At the same time it provided an opportunity to investigate simultaneously aspects of the hepatic and peripheral metabolism of carbohydrate and lipid in diabetes.

## METHODS.

Details on housing, feeding and handling of the baboons have been described in Chapter Three.

All baboons were fasted overnight. A single dose of Phencyclidine (Sernylan : Parke-Davis) 2 mg/kg was injected intramuscularly and this produced adequate sedation for handling, weighing, blood sampling and injection of the drug. Streptozotocin (Upjohn : Michigan) was weighed and dissolved in 20 ml sodium citrate buffer, pH 4.5, just prior to injection. The total calculated dose was then rapidly injected, intravenously as a single bolus.

Blood glucose was estimated by the hexokinase : glucose-6-phosphate dehydrogenase method (Appendix One page 121). Plasma ketones were



detected semi-quantitatively by the sodium nitroprusside reaction using a commercial reagent strip (Ketostix : Ames).

The Diabetogenic Dose of Streptozotocin: Four baboons were used in preliminary experiments in order to establish the diabetogenic dose. The results of these preliminary experiments to establish the diabetogenic dose are presented in Table 4(1) below.

TABLE 4(1) STREPTOZOTOCIN : DOSE RESPONSE RELATIONSHIP.

BABOON	DOSE OF STREPTOZOTOCIN	FASTING BLOOD GLUCOSE (mmol/l)	
		BEFORE STREPTOZOTOCIN	2 DAYS AFTER STREPTOZOTOCIN
A1	30 mg/kg	5,00	4,72
A2	50 mg/kg	4,22	13,22
A3	40 mg/kg	4,88	6,11
A4	40 mg/kg	4,33	5,11

Streptozotocin in a dose of 40 mg/kg or less was not diabetogenic. A dose of 50 mg/kg produced hyperglycaemia (13,22 mmol/l after 48 hours) without elevation of plasma ketones in baboon A2. After a week the blood glucose level in this animal was 43,8 mmol/l and mild ketonaemia was present.

On the basis of these findings a dose of 50 mg/kg was considered to be the smallest dose likely to be diabetogenic in all baboons. This dose was administered to ten normal baboons after metabolic investigations had been completed.

## RESULTS.

The response to streptozotocin is presented in Table 4(2) below.

TABLE 4(2) FASTING BLOOD GLUCOSE LEVELS AND PLASMA KETONES,  
FOUR DAYS AFTER STREPTOZOTOCIN 50 mg/kg.

BABOON	BLOOD GLUCOSE (mmol/l)	PLASMA KETONES (AMES-KETOSTIX)
B1	25,08	+
B2	7,7	NIL
B3	20,8	+
B4	23,79	+
B5	1,28*	NIL
B6	34,12	+
B7	15,17	NIL
B8	26,86	+
B9	16,5	NIL
B10	31,2**	++

\* Blood Glucose Concentration After 24 Hours.

\*\* Blood Glucose Concentration and Plasma Ketones  
After 48 Hours.

Baboons B2 and B7, which showed mild fasting hyperglycaemia, remained well with only minimal symptoms such as polydipsia or polyuria, and no weight loss. Baboon B5 was found semiconscious the day after receiving streptozotocin. The blood glucose concentration was 1,28 mmol/l, and despite treatment with intravenous glucose the baboon failed to recover from severe hypoglycaemia and died 34 hours after receiving streptozotocin. Moderate hypoglycaemia, 24 hours after streptozotocin has been reported previously in the vervet monkey (White *et al.* 1974). Post mortem histological sections showed centrilobular zonal necrosis of the liver (usually seen with hepatotoxins), and an acute tubular necrosis of the kidney. There was thus generalized toxicity in this animal and the

immediate cause of death was probably severe hypoglycaemia.

Baboon B10 developed severe hyperglycaemia and ketoacidosis and died during the night, 72 hours after receiving streptozotocin.

The remaining baboons (B1, B3, B4, B6, B8 and B9) showed polydipsia, polyuria and weight loss which averaged 3,3 kg. The animals remained alert and well during the first week. However, after this time progressive deterioration was noted. Hyperglycaemia became severe, usually greater than 40 mmol/l eventually and ketonaemia invariably developed. Untreated, none of the animals survived more than fourteen days. Post mortem histological sections usually showed a decrease in the number of pancreatic islets and disintegration or degeneration of islet cells. The liver showed severe fatty infiltration while sections of the kidney were normal in appearance.

#### DISCUSSION.

The dose of streptozotocin which is diabetogenic shows species variation. In mice diabetes was produced with a dose of 175 mg/kg (Schein and Bates, 1968). In the rat and in the dog 50 mg/kg was diabetogenic (Rakieten *et al.* 1963), while in monkeys diabetes was produced in five out of seven animals with a dose of 45 mg/kg, and consistently in all animals with 60 mg/kg (Pitkin and Reynolds, 1970; White *et al.* 1974). The results of the present study show that in the baboon 50 mg/kg is consistently diabetogenic.

The ratio of lethal dose to diabetogenic dose of streptozotocin has been reported as 3 in the rat, 1,5 to 2 in the mouse and unity in the dog (Rerup, 1970). In this study on baboons the ratio was also unity, the diabetogenic dose being responsible for the death of nine out of eleven baboons. Sensitivity to streptozotocin varies greatly in the species studied thus far, and appears to be greatest in the dog and baboon. The margin between diabetogenic and lethal doses is highest in the rat and there are reports of spontaneous recovery from Streptozotocin Diabetes in

this species (Junod *et al.* 1969; Rakieten *et al.* 1976a).

In earlier studies on rats it was reported that streptozotocin produced a mild metabolic disturbance, that ketonuria was rare and mortality low (Arison *et al.* 1967; Junod *et al.* 1967). In a later study on rhesus monkeys much greater metabolic disturbance and higher mortality were described (Pitkin and Reynolds, 1970). Of the seven monkeys that became hyperglycaemic, three died of severe diabetes after 6-8 days and one died after 19 days from septicaemia, to which diabetes was contributory. Thus over half the animals died from causes directly attributable to Streptozotocin Diabetes.

In the baboons used in this study, loss of appetite, polydipsia, polyuria and weight loss were prominent. Hyperglycaemia was marked and progressive, usually reaching levels close to or greater than 40 mmol/l during the second week. Ketonaemia, if not present initially, invariably developed after a week. The blood lactate concentration was increased and plasma triglycerides, free fatty acids and glycerol were elevated (Chapter Seven). There was thus widespread metabolic disturbance following streptozotocin administration in the baboon.

This study on baboons provides further evidence of the wide species variation in the response to streptozotocin. There are few reports on the use of streptozotocin in primates. In the study on vervet monkeys (White *et al.* 1974) toxicity and mortality were reported as extremely low, while in the study on rhesus monkeys (Pitkin and Reynolds, 1970) more than half the animals died from causes which could be attributed to Streptozotocin Diabetes. In addition to variation in response in different species, there is also a great disparity in the individual response of animals of a particular species. In the study by Pitkin and Reynolds (1970) seven rhesus monkeys received 45 mg/kg of streptozotocin, of which two remained normoglycaemic while five showed hyperglycaemia and acidosis of varying degree. In the present study there was also wide variation in response to streptozotocin. Four days after streptozotocin 50 mg/kg the level of fasting hyperglycaemia was less than 10 mmol/l in one baboon, between 15 and 30 mmol/l in six baboons and greater than 30 mmol/l in two baboons, while in one baboon this dose resulted in

generalized streptozotocin toxicity and death in less than 36 hours.

Such wide variation in individual response is not easily explained. All animals received identical diets for several months prior to streptozotocin injection and differences in essential metabolic cofactors (such as niacin, a precursor of NAD) are unlikely. The streptozotocin used in all experiments came from the same batch and the method of preparation and administration was always identical. Obviously no two animals are the same and the genetic diversity within a species and the *biochemical individuality* of each animal are relevant to their variable response.

#### SUMMARY.

- (1) Streptozotocin, 50 mg/kg intravenously, is consistently diabetogenic in the baboon (*Papio ursinus*). The level of hyperglycaemia showed marked individual variation ranging from 8 to 34 mmol/l four days after streptozotocin.
- (2) The acute hyperglycaemia is progressive and ketonaemia invariably develops. Blood lactate, plasma triglycerides, free fatty acids and glycerol concentrations are significantly increased.
- (3) The baboon appears to be extremely sensitive to Streptozotocin Diabetes. Of nine animals that became diabetic seven died of severe uncontrolled diabetes within two weeks of receiving streptozotocin.
- (4) At post mortem the main histopathological features observed consistently were severe fatty infiltration of the liver, and decreased as well as disrupted or degenerate pancreatic islets.

## CHAPTER FIVE

## GLUCOSE KINETICS

Glucose metabolism was studied in the normal and diabetic baboon. Experiments were carried out to determine glucose turnover rates, the extent of glucose recycling, glucose pool and glucose space.

Ten healthy adult male baboons were used in this study. Details on housing, feeding, handling and sedation prior to the experiments have been described in Chapter Three, page 20. The experiments were carried out between 8 a.m. and 12 noon, after a 16-18 hour fast. After a priming dose, an infusion of [U-<sup>14</sup>C] D-glucose and [2-<sup>3</sup>H] D-glucose was commenced. The experimental procedure, analytical procedures and calculations have been described in Chapter Three, pages 21-23.

After all experiments were completed the animal was made diabetic with an intravenous injection of streptozotocin, 50 mg/kg (Chapter Four). Experiments were then repeated in the diabetic animal, 48 hours after the injection of streptozotocin in four baboons (B1, B4, B6 and B10), and 96 hours after streptozotocin in five baboons (B2, B3, B7, B8 and B9). Baboon B5 died 34 hours after streptozotocin and thus the effect of diabetes on glucose kinetics in this animal could not be determined.

The statistical significance of results after diabetes was determined by the *paired t-test* (thus the significance of values after diabetes was determined in relation to the normal values for each animal). Correlations were determined by the *Spearman rank* correlation coefficient.

## RESULTS.

All results, including those of previous studies, have been expressed in the International System of Units.

(1) Blood Glucose Specific Activities: The blood glucose specific activities and blood glucose concentration, of a representative study in which 50  $\mu\text{Ci}$  [ $U\text{-}^{14}\text{C}$ ] D-glucose and 75  $\mu\text{Ci}$  [ $2\text{-}^3\text{H}$ ] D-glucose were administered as a primed constant rate infusion in a normal baboon are shown in Figure 5.1.

In all the animals blood glucose concentration remained relatively constant during the infusion period, with occasional fluctuations of less than 0,5 mmol/l. The  $^{14}\text{C}$  and  $^3\text{H}$  blood glucose specific activities reached a steady state after ninety minutes. An occasional fluctuation after this time (e.g. the 120 minute value in Figure 5.1) was probably due to the experimental error of the method, or inherent biological fluctuation, or both. In the ten normal baboons studied the mean asymptotic  $^{14}\text{C}$  blood glucose specific activity ranged from 536 to 1686 dpm/ $\mu\text{mol}$ , while the mean  $^3\text{H}$  blood glucose specific activity at the asymptote ranged from 728 to 1251 dpm/ $\mu\text{mol}$ .

Figure 5.2 shows the blood glucose concentrations and blood glucose specific activities during a similar study in the same animal after the induction of Streptozotocin Diabetes. In the diabetic baboons the blood glucose concentration was also relatively constant during the infusion period, but fluctuations ranging up to 1,0 mmol/l sometimes occurred (e.g. the 180 minute value in Figure 5.2). The greater fluctuation in blood glucose concentration in diabetes is probably partly due to the greater experimental error in blood glucose determination in diabetic samples. (The SD of the differences in 38 pairs of duplicate analyses in the range 3,99 to 6,13 mmol/l was 0,06 while the SD in 35 pairs of duplicate analyses in the range 8,33 to 29,25 mmol/l was 0,41.) The blood glucose  $^{14}\text{C}$  and  $^3\text{H}$  specific activities reached a steady state after 90 minutes in all the diabetic baboons. An occasional fluctuation in the specific activity during the steady state (e.g. the 180 minute values in Figure 5.2) was, as in the normal study, also observed in the diabetic animal. In the nine diabetic baboons the mean asymptotic  $^{14}\text{C}$  blood glucose specific activity ranged from 233 to 832 dpm/ $\mu\text{mol}$  while the mean  $^3\text{H}$  blood glucose specific activity at the asymptote ranged from 240 to 1171 dpm/ $\mu\text{mol}$ .

(2) Glucose Turnover Rates: The Mean  $\pm$  S.E.M. of glucose turnover rates, before and after diabetes, are shown in Figure 5.3.

The glucose turnover rates in the ten normal baboons are shown in Figure 5.4. The blood glucose level (venous) in the normal animal was  $4,96 \pm 0,18$  mmol/l (Mean  $\pm$  S.E.M.). Using  $[U-^{14}C]$  D-glucose as the tracer the turnover rate ranged from 581 to 887  $\mu\text{mol/kg/hr}$  with a mean of  $719 \pm 35$ . The turnover rates calculated at the same time from the  $[2-^3H]$  D-glucose specific activity ranged from 677 to 1147  $\mu\text{mol/kg/hr}$  with a mean of  $904 \pm 50$ . The higher turnover rates with  $^3H$ -glucose were significantly different from the  $^{14}C$ -glucose turnover rates ( $p < 0,001$ ).

The glucose turnover rates in nine diabetic baboons are shown in Figure 5.5. The blood glucose level (venous) in the diabetic baboon was  $19,41 \pm 2,85$  mmol/l (Mean  $\pm$  S.E.M.). Using  $[U-^{14}C]$  D-glucose as the tracer the glucose turnover rates ranged from 944 to 3586  $\mu\text{mol/kg/hr}$  with a mean of  $2127 \pm 313$ . The glucose turnover rates calculated at the same time in the diabetic baboons from the  $[2-^3H]$  D-glucose specific activity ranged from 1172 to 4935  $\mu\text{mol/kg/hr}$  with a mean of  $2709 \pm 439$ . The higher turnover rates with  $^3H$ -glucose were significantly different from the  $^{14}C$ -glucose turnover rates ( $p < 0,005$ ).

Both the  $^{14}C$  and  $^3H$  glucose turnover rates in the diabetic baboons were significantly greater than the turnover rates before the induction of Streptozotocin Diabetes ( $p < 0,005$ ). Figure 5.6 summarises  $^{14}C$  and  $^3H$  turnover results before and after diabetes. It illustrates the significant increase in glucose turnover rates in all baboons after the production of diabetes, as well as the difference in turnover rates calculated from  $[U-^{14}C]$  D-glucose and  $[2-^3H]$  D-glucose specific activities, in normal and diabetic baboons.

No correlation was found between the glucose turnover rate and the presence of ketosis (Figure 5.7). A significant correlation was found between the glucose turnover rate and the blood glucose level (Figure 5.8) in the diabetic baboon.



(3) Glucose Recycling: The Mean and S.E.M. of glucose recycling, before and after diabetes, is shown in Figure 5.3.

Glucose recycling in the ten normal baboons and nine diabetic baboons is shown in Figure 5.9. In the normal baboons the amount of glucose recycled varied from 93 to 281  $\mu\text{mol/kg/hr}$  with a mean of  $184 \pm 20$  (Mean  $\pm$  S.E.M.). Thus on average recycling accounted for 20% of the normal turnover rate. After the production of Streptozotocin Diabetes glucose recycling ranged from 186 to 1348  $\mu\text{mol/kg/hr}$  with a mean of  $581 \pm 132$ . Recycling of glucose therefore accounted for 21% of the turnover rate after diabetes.

The increase in recycling after the production of diabetes was statistically significant ( $p < 0,02$ ). A direct relationship, which was statistically significant, was found between the glucose turnover rate and the amount of glucose recycled, before and after Streptozotocin Diabetes (Figure 5.10).

(4) Glucose Pool: The Mean and S.E.M. of glucose pool size, before and after diabetes, is shown in Figure 5.11.

Glucose pool in ten normal and eight diabetic baboons is shown in Figure 5.12. In the normal baboons the glucose pool ranged from 25,8 to 62,5  $\text{mmol}$  with a mean of  $43,8 \pm 3,8$  (Mean  $\pm$  S.E.M.). After producing diabetes the glucose pool ranged from 66,7 to 204  $\text{mmol}$  with a mean of  $117 \pm 15,7$ . Expressed as a function of body weight the glucose pool increased from  $1,5 \pm 0,1$   $\text{mmol/kg}$  (Mean  $\pm$  S.E.M.) to  $4,6 \pm 0,6$   $\text{mmol/kg}$  after diabetes. The increase in glucose pool after diabetes was statistically significant ( $p < 0,001$ ).

There was a significant positive correlation between the glucose pool ( $\text{mmol/kg}$ ) and the glucose turnover rate ( $\mu\text{mol/kg/hr}$ ) in the normal as well as in the diabetic baboon (Figure 5.13).

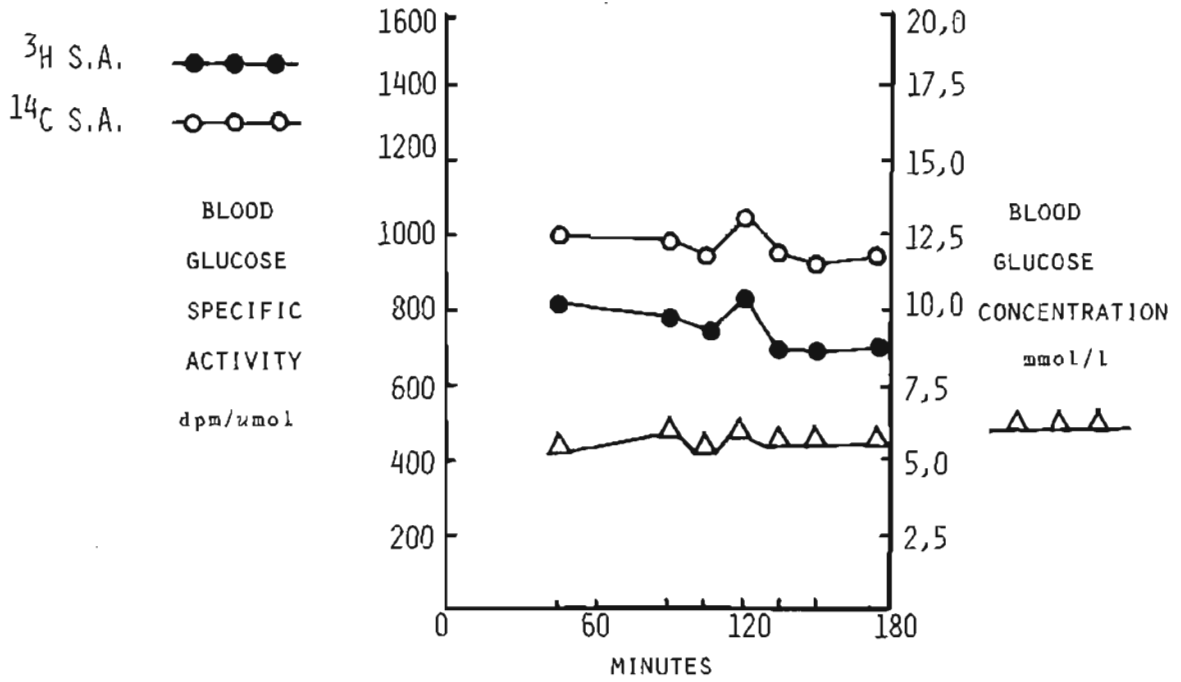


FIGURE 5.1 : BLOOD GLUCOSE CONCENTRATIONS;  $^{14}\text{C}$  AND  $^3\text{H}$  SPECIFIC ACTIVITY-TIME CURVES IN BABOON B4, BEFORE **DIABETES**.

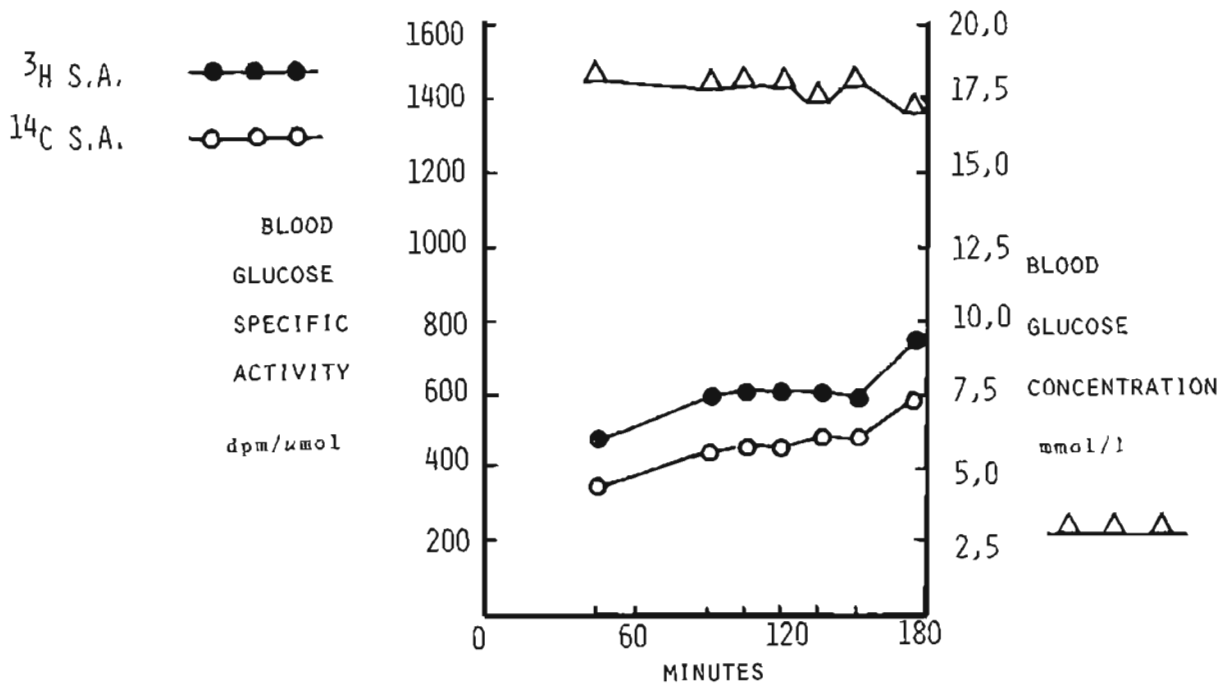


FIGURE 5.2 : SAME AS FIGURE 5.1; BABOON B4, AFTER **DIABETES**.

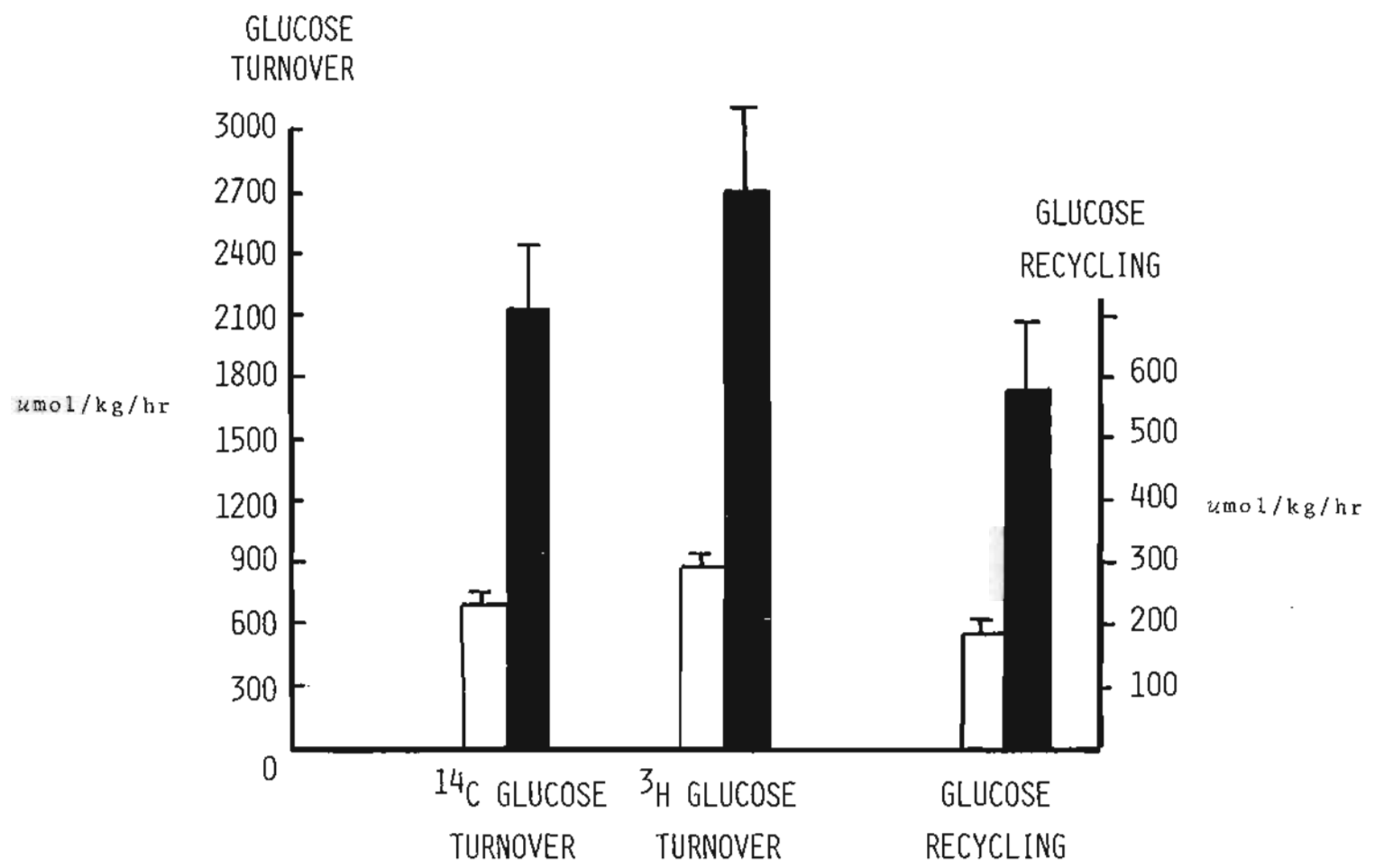


FIGURE 5.3 : GLUCOSE TURNOVER RATES AND GLUCOSE RECYCLING. VALUES REPRESENT MEAN  $\pm$  S.E.M.

NORMAL  DIABETIC

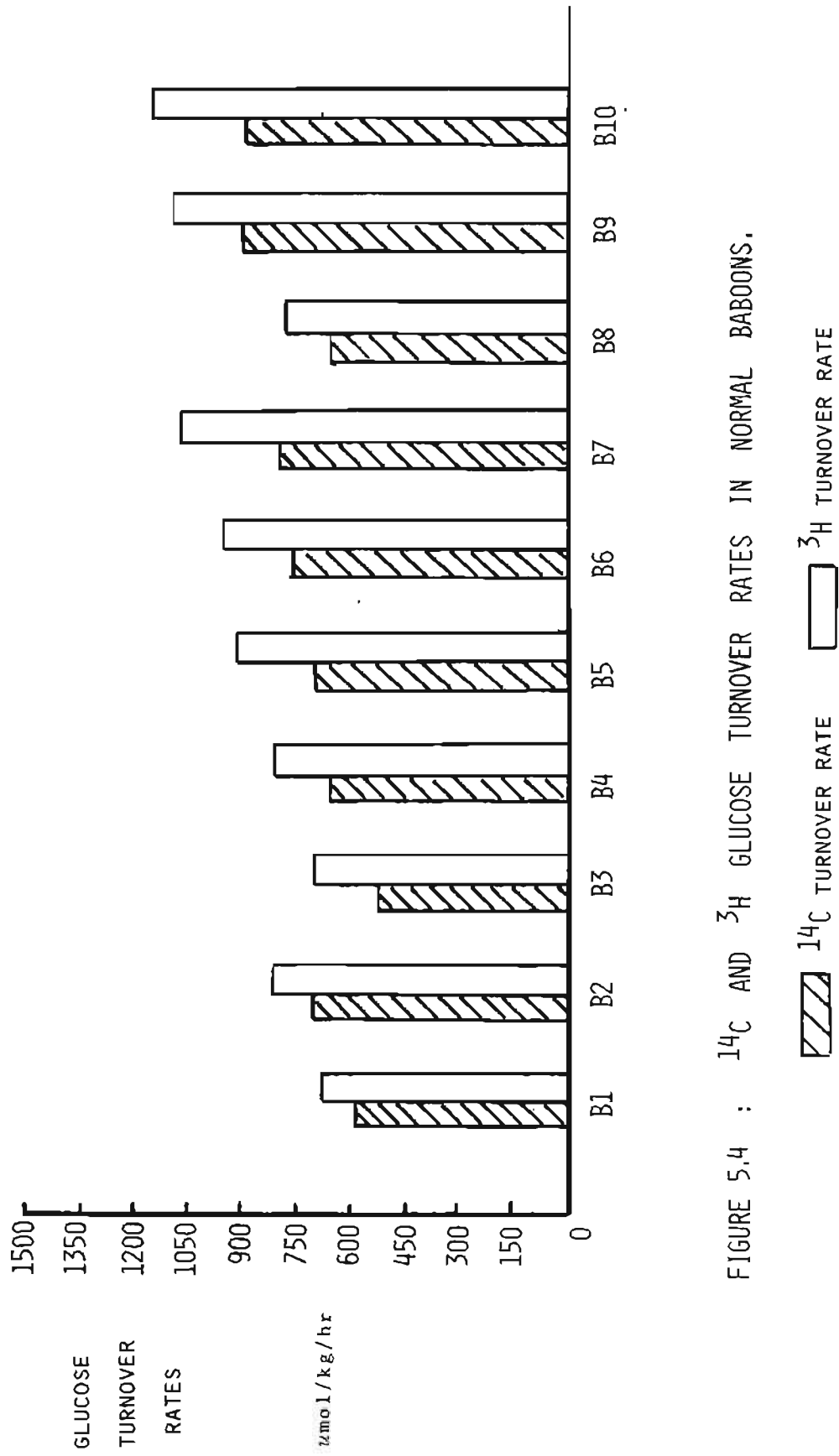
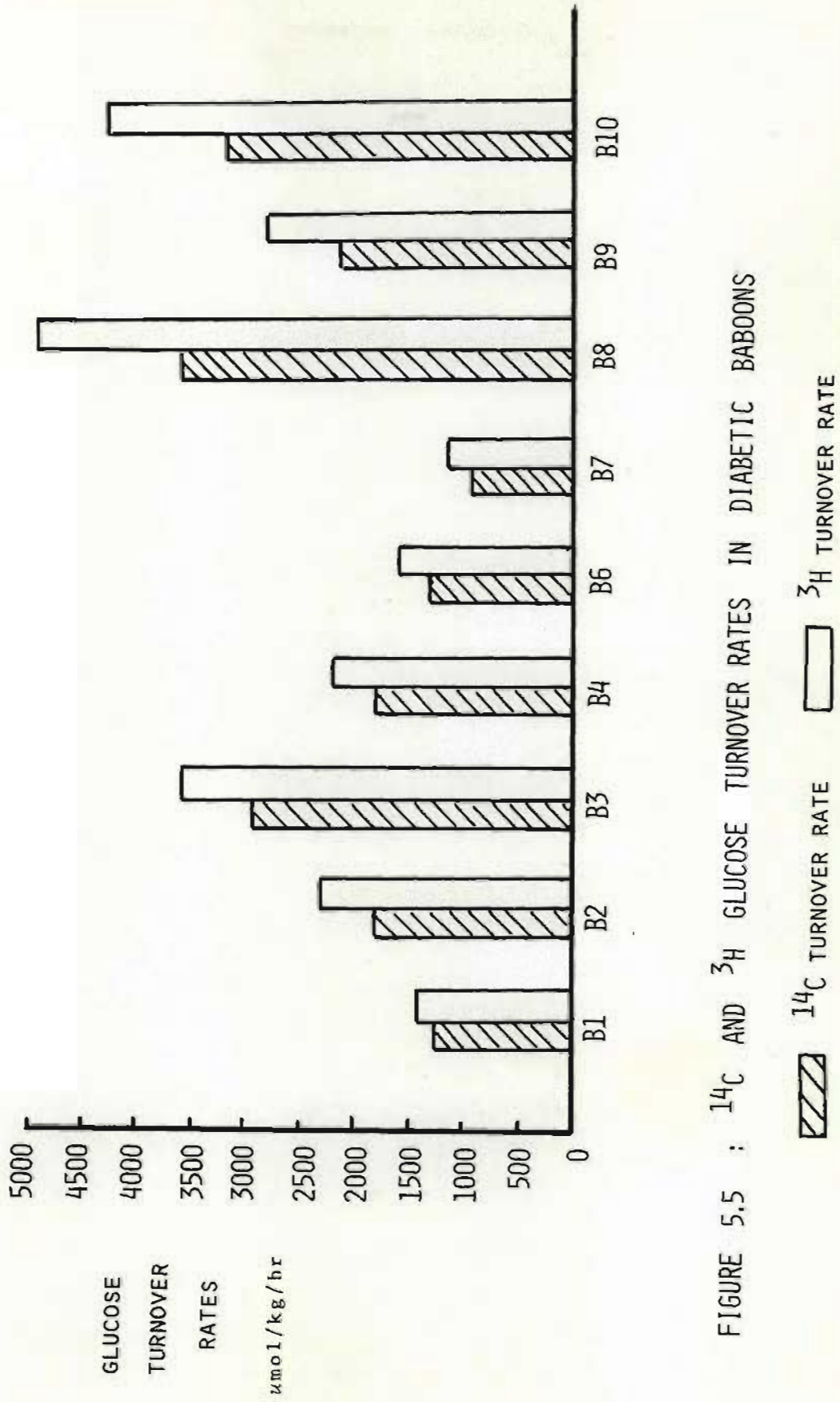


FIGURE 5.4 :  $^{14}\text{C}$  AND  $^3\text{H}$  GLUCOSE TURNOVER RATES IN NORMAL BABOONS.

FIGURE 5.5 :  $^{14}\text{C}$  AND  $^3\text{H}$  GLUCOSE TURNOVER RATES IN DIABETIC BABOONS

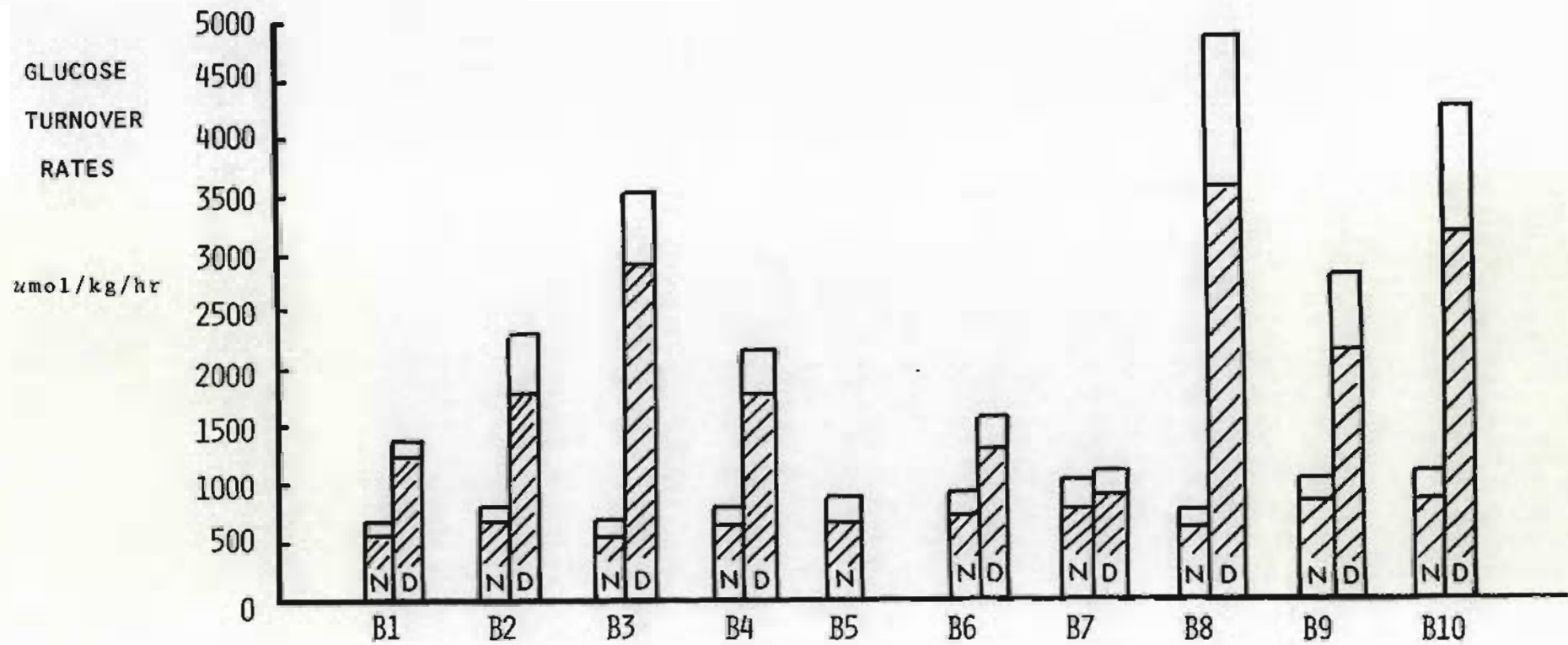




FIGURE 5.6 :  $^{14}\text{C}$  AND  $^3\text{H}$  GLUCOSE TURNOVER RATES BEFORE AND AFTER DIABETES.

N - NORMAL  
D - DIABETIC

  $^{14}\text{C}$  TURNOVER RATE  
  $^3\text{H}$  TURNOVER RATE

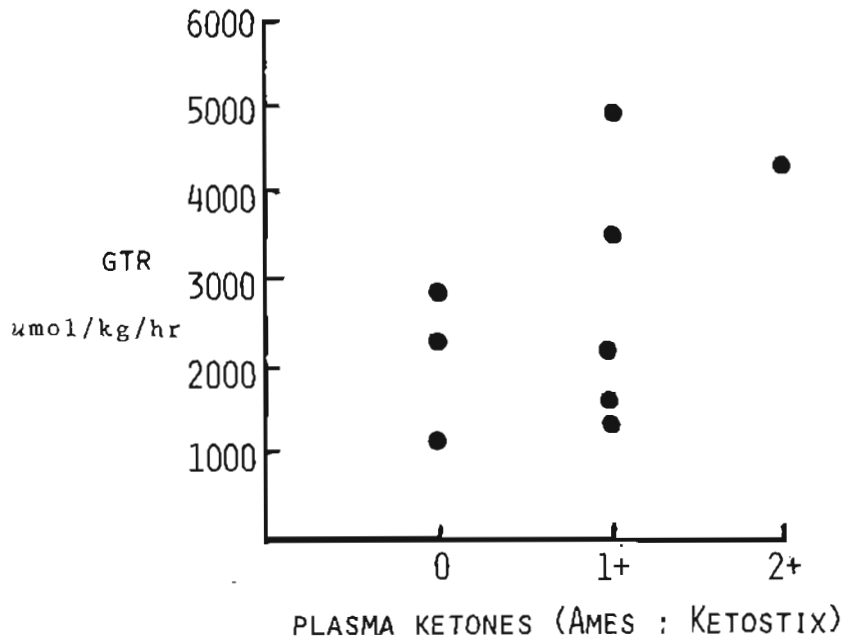


FIGURE 5.7 : CORRELATION BETWEEN GLUCOSE TURNOVER RATE (GTR) AND PLASMA KETONES IN THE DIABETIC BABOON

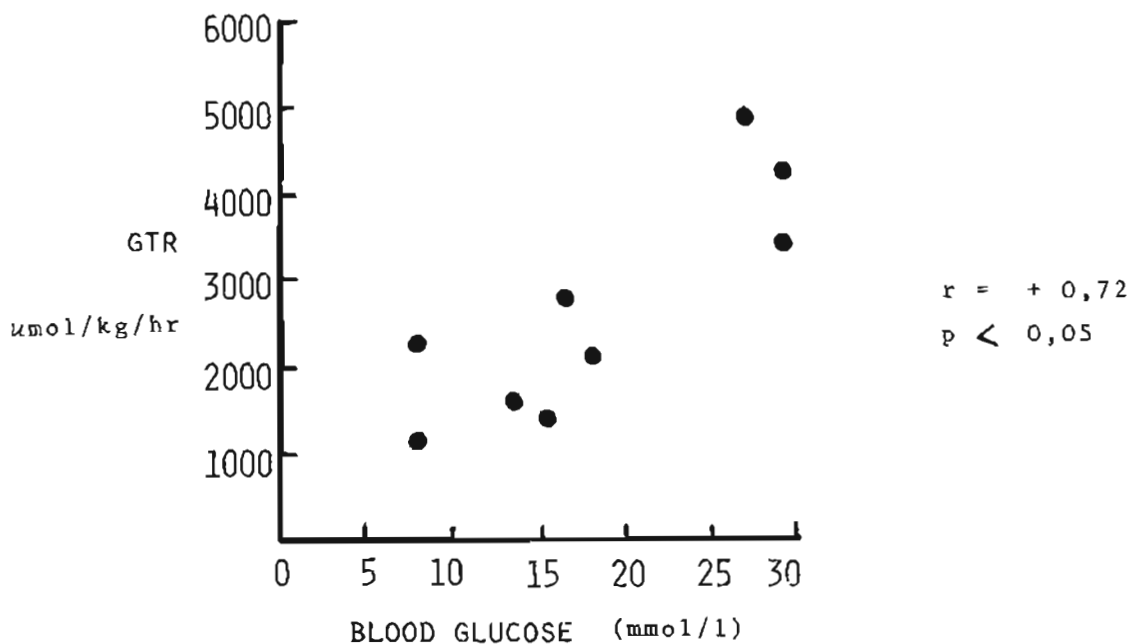


FIGURE 5.8 : CORRELATION BETWEEN GLUCOSE TURNOVER RATE (GTR) AND BLOOD GLUCOSE CONCENTRATION IN THE DIABETIC BABOON

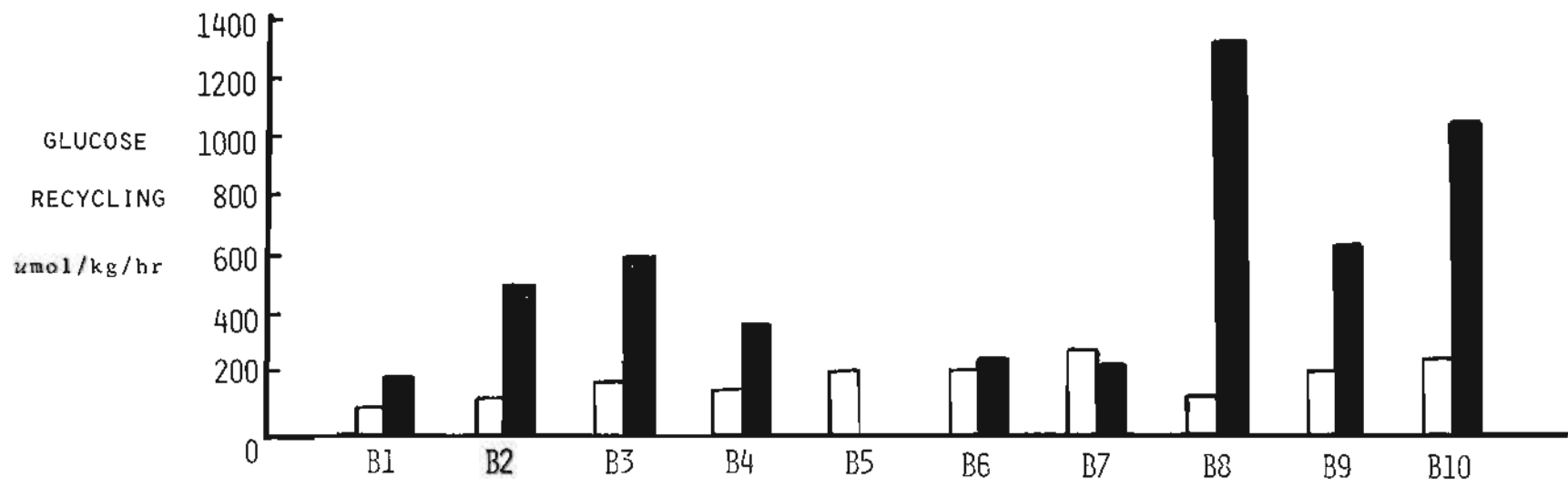


FIGURE 5,9 : GLUCOSE RECYCLING BEFORE AND AFTER DIABETES

NORMAL       DIABETIC



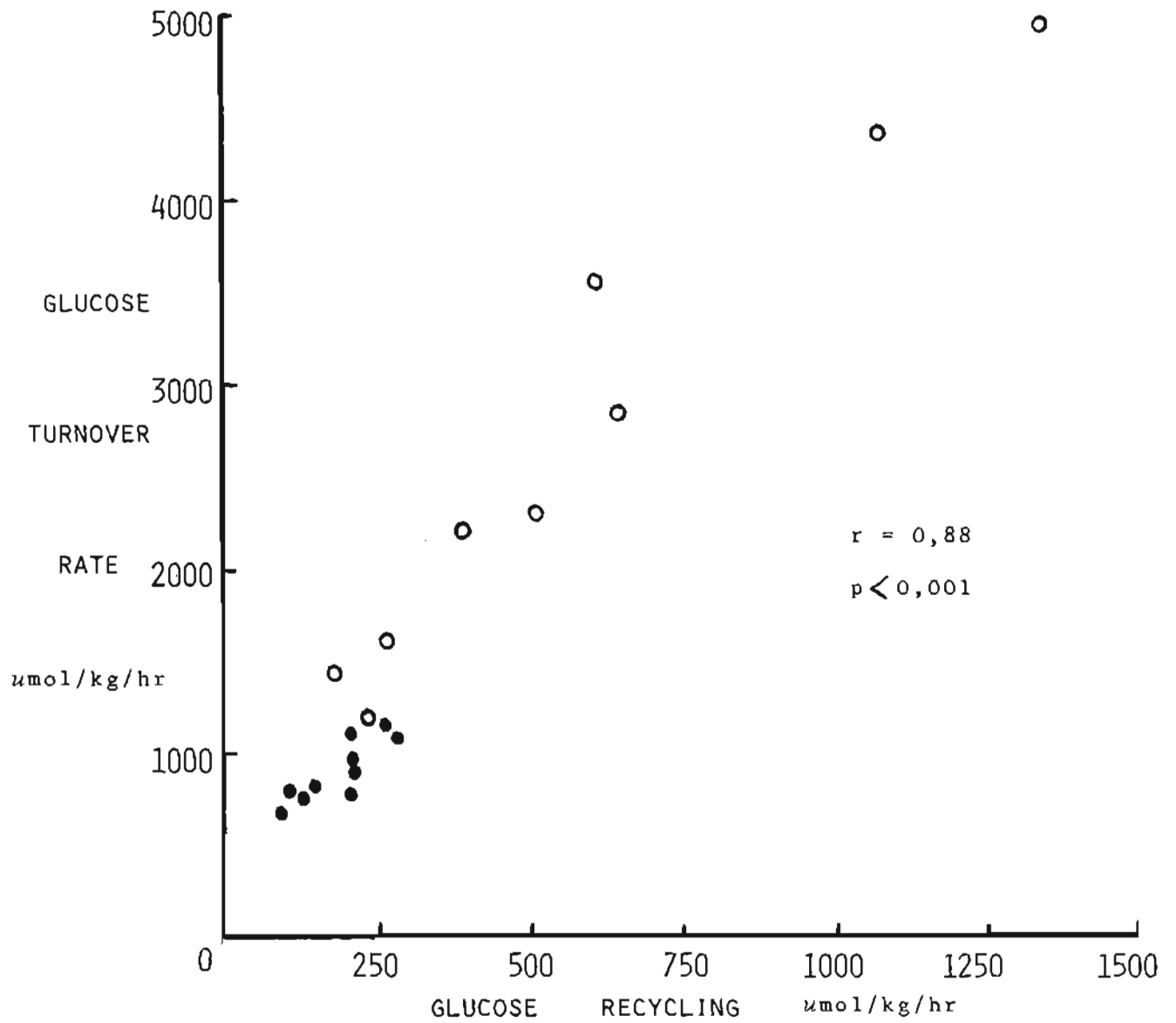


FIGURE 5.10 : CORRELATION BETWEEN GLUCOSE TURNOVER RATE AND GLUCOSE RECYCLING IN NORMAL (•) AND DIABETIC (○) BABOONS.

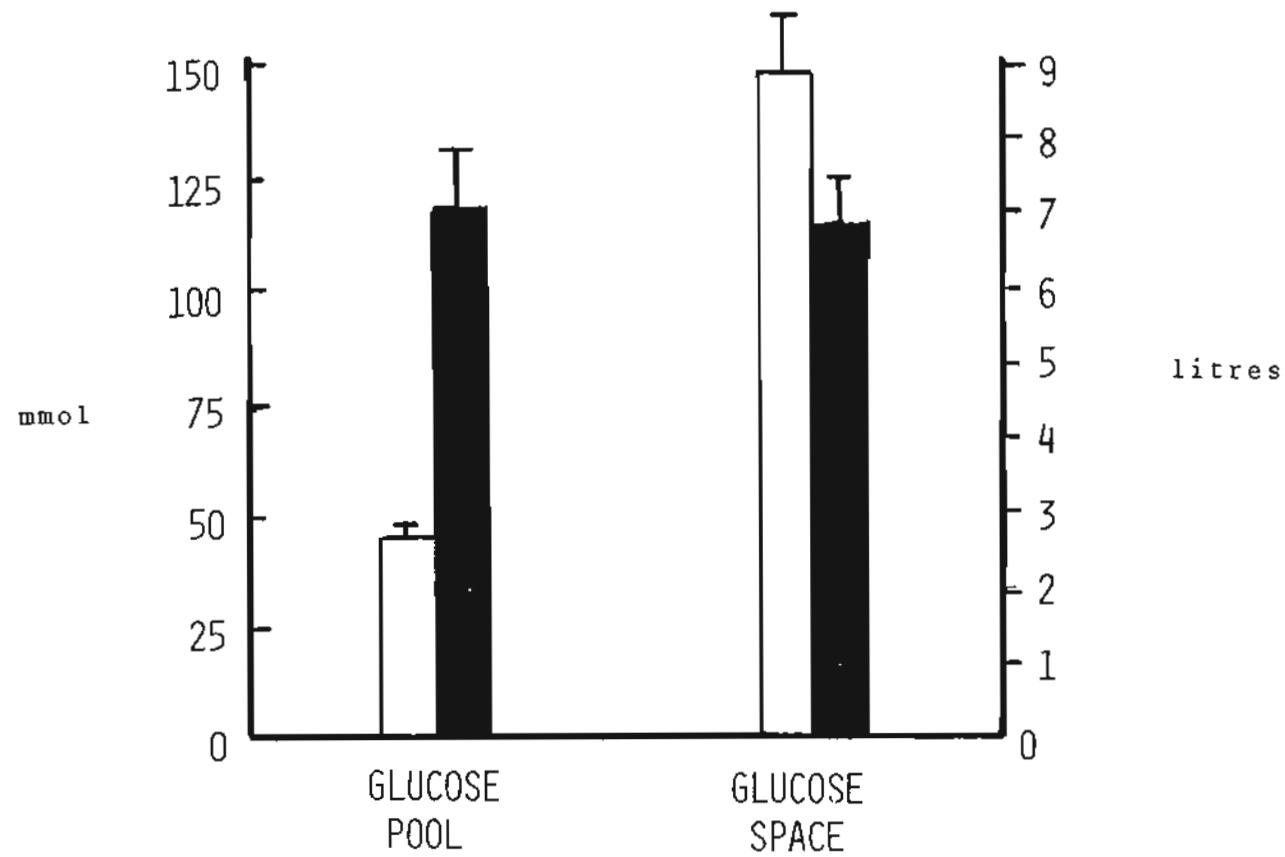


FIGURE 5.11 : GLUCOSE POOL AND GLUCOSE SPACE.  
VALUES REPRESENT MEAN  $\pm$  S.E.M.

NORMAL  DIABETIC

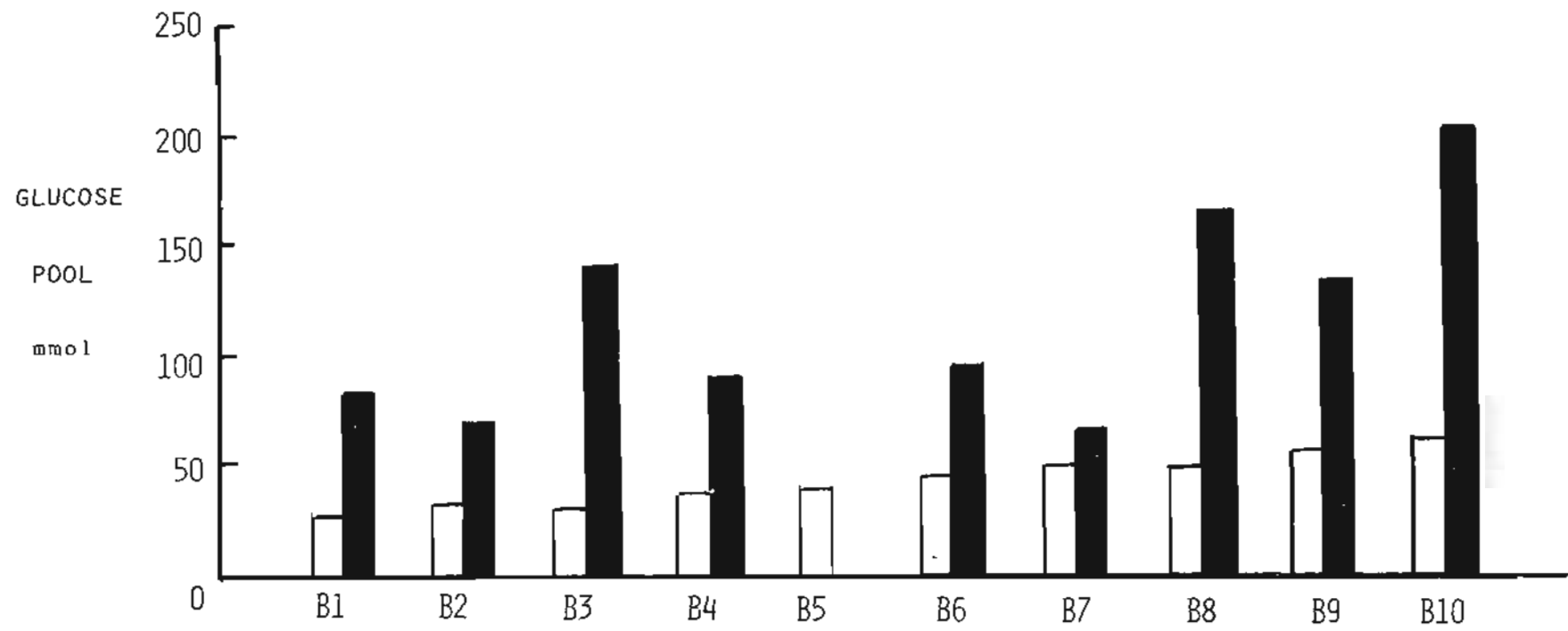



FIGURE 5.12 : GLUCOSE POOL BEFORE AND AFTER DIABETES.

NORMAL 

DIABETIC 

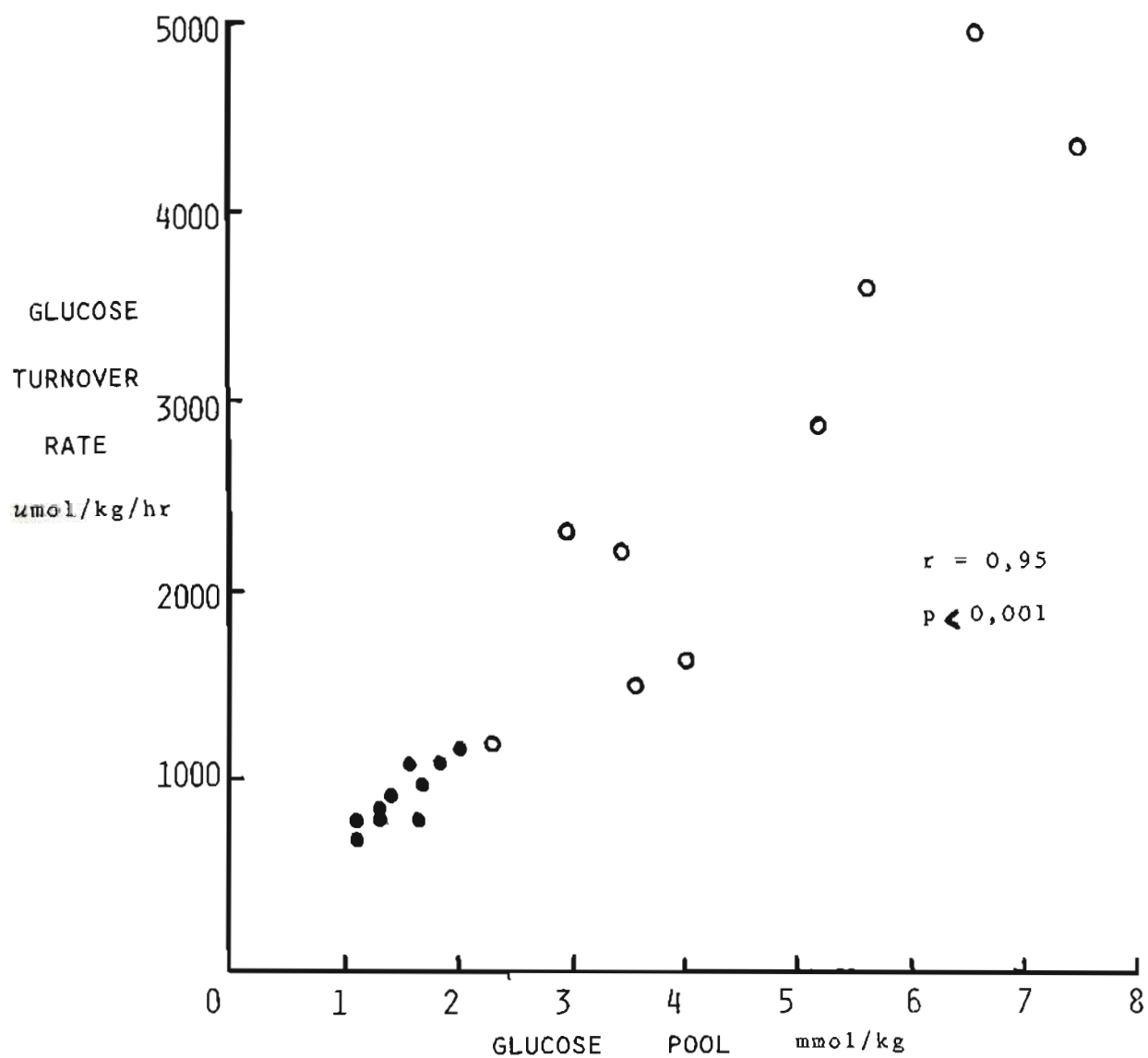


FIGURE 5.13 : CORRELATION BETWEEN GLUCOSE TURNOVER RATE AND GLUCOSE POOL IN NORMAL (•) AND DIABETIC (○) BABOONS.

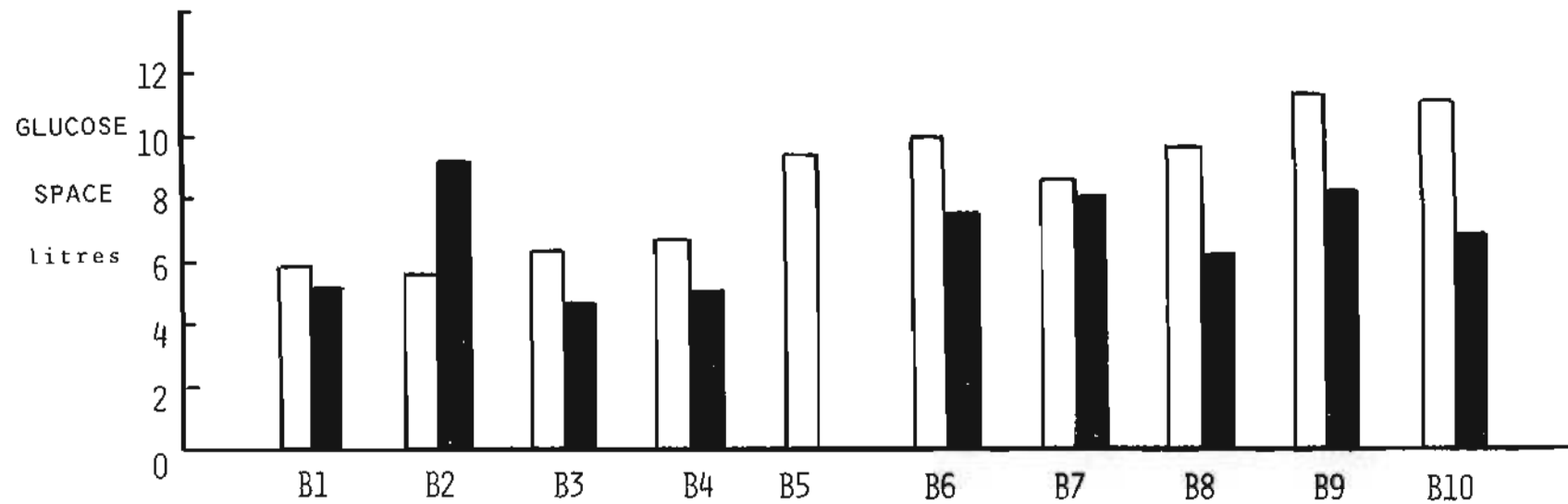


FIGURE 5.14 : GLUCOSE SPACE BEFORE AND AFTER DIABETES.

NORMAL       DIABETIC

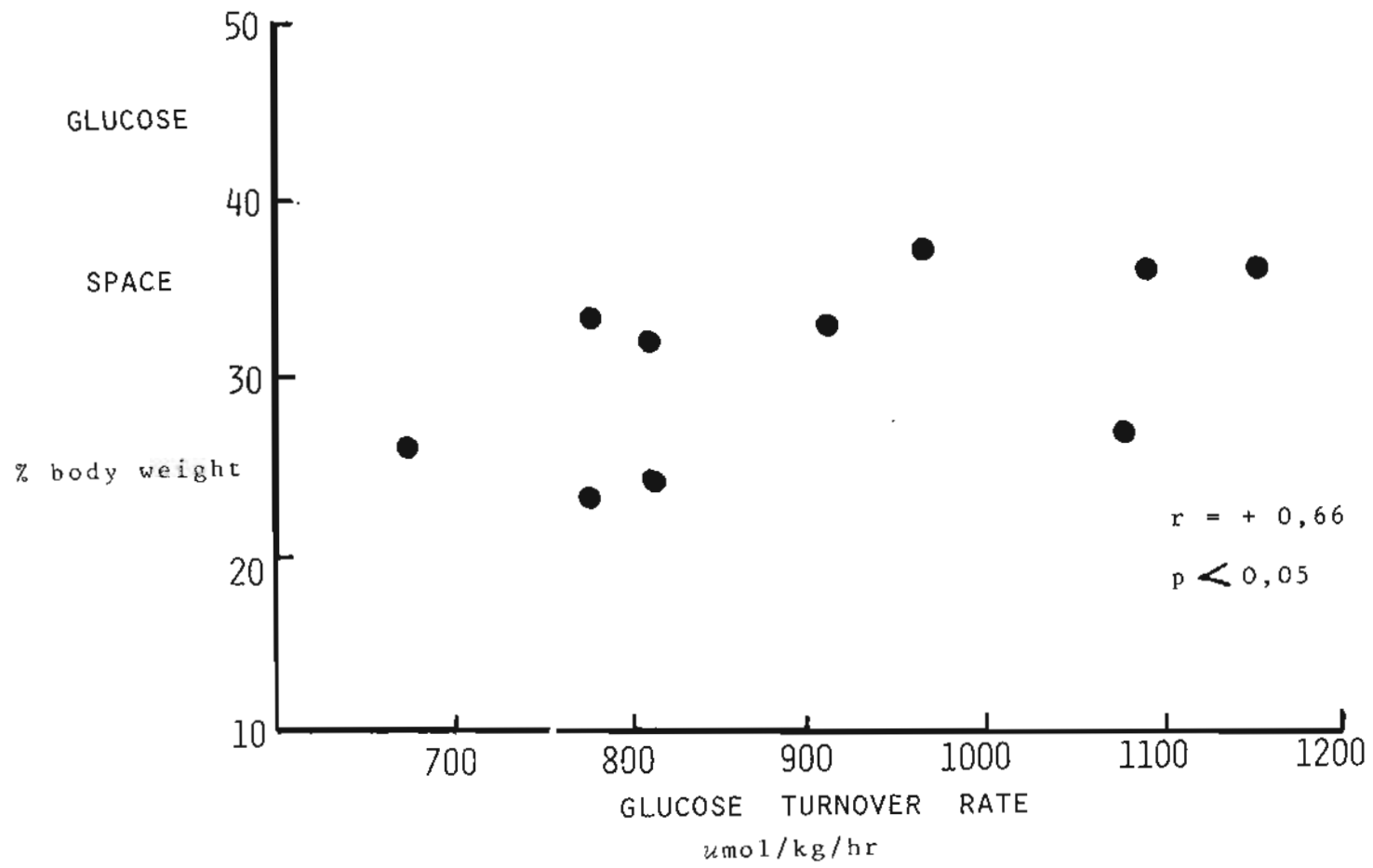


FIGURE 5.15 : CORRELATION BETWEEN GLUCOSE SPACE AND GLUCOSE TURNOVER RATE IN THE NORMAL BABOON

(5) Glucose Space: The Mean  $\pm$  S.E.M. of glucose space, before and after diabetes, is shown in Figure 5.11.

Glucose space in ten normal and nine diabetic baboons is shown in Figure 5.14. The glucose space in the normal baboons ranged from 5,9 to 11,4 litres with a mean of  $8,8 \pm 0,6$  (Mean  $\pm$  S.E.M.). After diabetes the glucose space ranged from 4,7 to 9,2 litres with a mean of  $6,8 \pm 0,5$ . The decrease in glucose space after diabetes was statistically significant ( $p < 0,02$ ).

Expressed as a fraction of body weight the glucose space decreased from a mean of 31% to 26% after diabetes. A significant positive correlation was found between the glucose turnover rate ( $\mu\text{mol/kg/hr}$ ) and the glucose space (% body weight) in the normal baboon (Figure 5.15). A statistically significant correlation was not found in the diabetic baboon.

## DISCUSSION.

### Glucose Kinetics in Normal Baboons

The mean asymptotic specific activities varied considerably, the highest being about two to three times the lowest. The asymptotic value attained is determined by the interaction of several factors. Two factors of importance are the size of the glucose pool, which determines the extent of dilution of the tracer glucose, and the glucose turnover rate which indicates the rate at which glucose (and hence tracer glucose) is being metabolised. Thus the asymptotic specific activity was found to be lower in those baboons with larger glucose pools and higher turnover rates. This was especially evident after the production of diabetes where as a result of an increase in turnover rates and glucose pool the asymptotic specific activities were significantly lower than corresponding values before diabetes.

Glucose Turnover Rates and Recycling: As glucose kinetics have not been studied previously in baboons it makes direct comparison of the results of this study with other studies difficult. However, Koerker *et al.* (1974), while studying the effects of somatostatin on the endocrine pancreas of the baboon, performed three experiments to determine glucose turnover rates. They administered [U- $^{14}\text{C}$ ] D-glucose as a primed infusion to overnight fasted, conscious, chair restrained male baboons and found an average glucose turnover rate of 1167  $\mu\text{mol/kg/hr}$  (767-1567  $\mu\text{mol/kg/hr}$ ). This is more than 60% higher than the mean [U- $^{14}\text{C}$ ] D-glucose turnover rate of 719  $\mu\text{mol/kg/hr}$  in the present study. Considerable individual variation was also noted in the present study with the  $^{14}\text{C}$ -glucose turnover rates ranging from 581 to 887  $\mu\text{mol/kg/hr}$ .

Koerker *et al.* (1974) studied conscious baboons, restrained in a chair. It is very likely that the baboon would be, not infrequently, straining against the physical restraints imposed upon it. This being equivalent to exercise of varying intensity and duration during the experiment, would result in higher glucose turnover rates (Wahren *et al.* 1971). Furthermore, a sedated baboon, as in the present study, has decreased glucose requirements and consequently hepatic glucose production would be lower in response to diminished utilization. Additional factors that may be relevant to the marked difference in result between the two studies are the species and genetic background of the baboons, and the diet and handling of the animals prior to the experiments. In addition, as only three experiments were performed by Koerker *et al.*, it is possible that the mean value they obtained is not truly representative.

In the absence of other studies on baboons the only possible comparison at present is between results of the present study on baboons and the results of experiments in other species where similar techniques have been used. Table 5(1) below summarises the results of experiments in the rabbit, rat, dog and man in which  $^3\text{H}$ -glucose and/or  $^{14}\text{C}$ -glucose were administered as a primed infusion after an overnight fast.



TABLE 5(1) NORMAL GLUCOSE TURNOVER RATES AND RECYCLING IN THE RAT, RABBIT, DOG, BABOON AND MAN.

REFERENCE	SPECIES	GLUCOSE TRACER	NUMBER STUDIED	TURNOVER RATE $\mu\text{mol/kg/hr}$	RECYCLING Fraction of Turnover Rate
Katz <i>et al.</i> 1974b	Rat	[U- <sup>14</sup> C] and [2- <sup>3</sup> H]	3	<sup>14</sup> C : 2416 <sup>3</sup> H : 3516	31-36%
Katz <i>et al.</i> 1974b	Rabbit	[U- <sup>14</sup> C] and [2- <sup>3</sup> H]	7	<sup>14</sup> C : 978 <sup>3</sup> H : 1328	26%
Issekutz <i>et al.</i> 1972	Dog	[U- <sup>14</sup> C] and [2- <sup>3</sup> H]	3	<sup>14</sup> C : 794 <sup>3</sup> H : 1011	24%
Forbath and Hetenyi, 1970	Dog	[U- <sup>14</sup> C]	6	1033	-
Kreisberg <i>et al.</i> 1970	Man	[1- <sup>14</sup> C]	5	683	10,4%
Paul and Bortz, 1969	Man	[U- <sup>14</sup> C]	3	672	-
Present Study	Baboon	[U- <sup>14</sup> C] and [2- <sup>3</sup> H]	10	<sup>14</sup> C : 719 <sup>3</sup> H : 904	20%

Examination of the results in Table 5(1) reveals wide variation in glucose turnover rates and recycling in different species. Turnover rates and recycling are highest in the rat and lowest in man. In the present study the mean <sup>3</sup>H-glucose turnover rate in ten normal baboons was

904  $\mu\text{mol/kg/hr}$ , with a range from 677 to 1147  $\mu\text{mol/kg/hr}$ . Thus considerable individual variation in turnover rate was present. This is probably because of inherent biological differences between the animals and also because of the difficulty in achieving an identical dietary *intake* in all animals (although the diet provided to each was similar). Furthermore, the amount of spontaneous physical activity prior to the experiment varied widely and thus it is likely that the "metabolic status" of each animal differed accordingly at the beginning of the experiment.

The average amount of glucose recycled was 184  $\mu\text{mol/kg/hr}$ . This meant that about 20% of the mean glucose turnover rate was probably derived from lactate. The glucose turnover rates and glucose recycling in the baboon are intermediate between that reported in man and in the dog. It is reassuring to note that the results of the present study integrate satisfactorily with the spectrum of turnover rates and recycling reported in other species, from the rat to man.

A direct relationship was noted between the glucose turnover rate and the amount of glucose recycled (Figure 5.10). However, although animals with higher turnover rates showed greater recycling, the fraction of the turnover rate recycled did not differ significantly in the ten normal baboons ( $20 \pm 2\%$ ).

When  $[\text{U-}^{14}\text{C}]$  D-glucose was used as the tracer it underestimated the glucose turnover rate by 31% in the rat and 26% in the rabbit (Katz *et al.* 1974b), and 24% in the dog (Issekutz *et al.* 1972). In baboons  $[\text{U-}^{14}\text{C}]$  D-glucose underestimated the glucose turnover rate by an average of 20%. The degree of underestimation is a function of the fraction of the turnover rate recycled. Thus in the rat, where recycling accounts for one third of the turnover rate, underestimation of the turnover rate by up to 31% occurs when  $[\text{U-}^{14}\text{C}]$  D-glucose is the tracer. In man, where recycling is about one third that in the rat, underestimation of the turnover rate is in the region of 10-15%.

It is of interest to compare estimates of hepatic glucose production by the direct arterial-hepatic vein catheterisation technique with estimates

by the (indirect) tracer technique. In two recent studies on 12-14 hour fasted normal human subjects the hepatic glucose production, measured by the catheterisation technique, was 667 to 683  $\mu\text{mol/kg/hr}$  (Wahren *et al.* 1977; Liljenquist *et al.* 1977). Thus certainly in man, there appears to be close agreement in estimates of glucose turnover rate between the catheterisation and tracer techniques.

Glucose Pool: The mean total glucose pool in the normal baboon was 43,8 mmol. Expressed as a function of body weight the mean glucose pool was 1,5 mmol/kg. Estimates of glucose pool in man show considerable variation. In two studies using similar techniques as in the present study, the mean glucose pool in three normal subjects was 1,0 mmol/kg (Paul and Bortz, 1969), and 1,6 mmol/kg in five normal subjects (Kreisberg *et al.* 1970). The glucose pool determined in the baboon is thus in close agreement with the results of the latter study in man. They also compare favourably with the results of two other studies in normal man (using the single dose tracer technique) in which the glucose pool was 1,5 mmol/kg (Manougian *et al.* 1964) and 1,3 mmol/kg (Shames *et al.* 1971). In two studies on dogs, using similar techniques as in the present study, the glucose pool was reported to be 1,1 to 1,4 mmol/kg in one study (Forbath and Hetenyi, 1970) and 1,3 to 1,9 mmol/kg in the other study (Steele *et al.* 1956).

There was a significant positive correlation between the glucose pool (mmol/kg) and the glucose turnover rate ( $\mu\text{mol/kg/hr}$ ) (Figure 5.13). It is not surprising to find that a relationship exists between the rate of production and utilization of glucose (i.e. the turnover rate) and the quantity of glucose that is present (i.e. the glucose pool). The balance between glucose pool size and glucose production and utilization in the normal animal depends on its ability to alter insulin secretion appropriately when changes occur in the glucose pool.

Glucose Space: The mean glucose space in the normal baboon was 8,8 litres. As a fraction of body weight, the glucose space represented on average

31%. These results are similar to values determined in normal man, in whom glucose space was reported as being 32% (Manougian *et al.* 1964), 28% (Forbath and Hetenyi, 1966), and 33% (Shames *et al.* 1971). In the normal dog glucose space has been reported as 21-31% of body weight in one study (Steele *et al.* 1956), and 24-33% in another study (Ninomiya *et al.* 1965). Thus it is apparent that, when expressed as a function of body weight, glucose space and glucose pool values do not differ greatly in the dog, baboon and man.

As with the glucose pool, there was also a significant correlation between glucose space and the glucose turnover rate (Figure 5.15). There is no satisfactory explanation for the observed relationship. If a higher turnover rate was associated with a larger space because it resulted in a larger pool then a correlation between glucose pool and glucose space would be expected. This was not found in the present study. However, it is possible that a greater rate of utilization (as indicated by higher turnover rate) is associated with increased intracellular distribution of glucose and hence an apparently greater glucose space.

#### Glucose Kinetics in Diabetic Baboons

Streptozotocin Diabetes resulted in abnormalities in glucose kinetics in all the baboons studied.

Glucose Turnover Rates: Glucose turnover rates increased dramatically after producing diabetes. The mean turnover rate of 2709  $\mu\text{mol/kg/hr}$  in the diabetic baboon was three times greater than the mean turnover rate in the normal baboon. This meant that in the diabetic animal there was a threefold increase in hepatic glucose production. These results are especially significant because glucose production rates were determined in each baboon prior to it becoming diabetic. The results of this study indicate unequivocally that in acute Streptozotocin Diabetes, which is characterized by insulin deficiency, hepatic overproduction of glucose is a very significant factor in the pathogenesis of hyperglycaemia. Glucose overproduction was observed in all animals, whether studied at 48 or 96 hours after streptozotocin.

Increased glucose production in diabetes has been reported in previous studies. In four insulin deprived pancreatectomised dogs (mean blood glucose 19,86 mmol/l) the mean glucose production was 1883  $\mu\text{mol/kg/hr}$ , compared to 939  $\mu\text{mol/kg/hr}$  before diabetes (Forbath and Hetenyi, 1970). In an earlier study by the same workers on human subjects, the mean glucose production rate was 661  $\mu\text{mol/kg/hr}$  in six normal subjects, 872  $\mu\text{mol/kg/hr}$  in sixteen nonketotic diabetic subjects and 1233  $\mu\text{mol/kg/hr}$  in four ketotic diabetic subjects (Forbath and Hetenyi, 1966). In maturity-onset diabetic subjects (mean blood glucose 11,9 mmol/l) glucose production rates were reported to be about three times greater than in a control group of healthy subjects (Bowen and Moorhouse, 1973).

In contrast to these reports of increased endogenous glucose production in diabetes, including the results of the present study, there are also several reports of normal glucose production rates in diabetic subjects (Reichard *et al.* 1961; Manougian *et al.* 1964; and Wahren *et al.* 1972). These discrepancies are probably due to differences in methodology and also to the considerable variation in the type and severity of the diabetes in the groups of subjects studied. The study by Reichard *et al.* (1961) was confined to elderly subjects with mild diabetes, while in the study by Manougian *et al.* (1964) some diabetic subjects had breakfast and antidiabetic treatment before the experiments. The insulin dependent subjects studied by Wahren *et al.* (1972) had mild to moderate hyperglycaemia (mean blood glucose 13,85 mmol/l) and a residual effect from the last dose of insulin cannot be excluded.

It must be emphasized that while in some studies on diabetic subjects glucose production rates were reported to be not increased, they are nevertheless grossly abnormal. Elevation of blood glucose concentration in a normal person (e.g. an increase of about 1 mmol/l by a constant rate infusion of glucose) results in marked inhibition of hepatic glucose production because of glucose induced insulin release (Felig and Wahren, 1971). Thus, relative to the ambient glucose concentration, a "normal" glucose production rate in a diabetic is in fact abnormal.

It has been suggested in previous reports that the degree of overproduction

of glucose is related to the severity of the diabetes. Thus while endogenous glucose production may be "normal" or slightly increased in mild diabetes, overproduction would be greatest in severe diabetes, particularly when ketosis is present. The results of the present study showed that while glucose overproduction correlated significantly with the degree of hyperglycaemia there was no correlation with the presence or absence of ketosis (Figures 5.7 and 5.8). A similar observation was made by Forbath and Hetenyi (1966) in their study of ketotic and nonketotic diabetic subjects.

In normal baboons a relationship between fasting blood glucose levels and glucose production rate was also apparent. The mean blood glucose level of the three normal baboons with the highest turnover rates (mean of 1104  $\mu\text{mol/kg/hr}$ ) was 5,51 mmol/l, while in the three normal baboons with the lowest turnover rates (mean of 746  $\mu\text{mol/kg/hr}$ ) it was 4,74 mmol/l. However the correlation between glucose turnover rate and blood glucose level in the normal baboon was not statistically significant ( $r = + 0,44$ ;  $p < 0,1$ ).

Glucose Recycling: The increased glucose turnover rates in diabetic baboons were associated with a more than threefold increase in the amount of glucose recycled. As in the normal baboons, the extent of recycling correlated with the turnover rates (Figure 5.10). This meant that the higher turnover rates in diabetes were associated with increased formation of glucose from lactate. However, the fraction of the turnover rate derived from lactate in the diabetic baboon (21%) was not significantly different from that in the normal animal (20%). In a study on rats (Baker *et al.* 1961) it was similarly reported that, although the amount of glucose recycled had increased twofold after diabetes, the fraction of the turnover rate recycled was not significantly increased (from 32% to 34% after diabetes).

Glucose Pool: After the production of Streptozotocin Diabetes the glucose pool increased nearly threefold. Expressed as a function of body weight glucose pool increased from a mean of 1,5 mmol/kg body weight in normal baboons to 4,6 mmol/kg in diabetic baboons. Similar results have been

reported in other studies. In diabetic nonketotic subjects the glucose pool was reported to be more than twice that in normal subjects, while in ketotic diabetics it was more than three times greater than in normal subjects (Forbath and Hetenyi, 1966). In the study by Manougian *et al.* (1964) the mean glucose pool was reported to be 1,5 mmol/kg in normal subjects and 4,5 mmol/kg in diabetic subjects. However, some subjects were not fasted and some had received oral antidiabetic therapy prior to the study. In an earlier study on pancreatectomised dogs (Searle *et al.* 1954) the glucose pool was found to be increased about threefold in diabetic dogs in comparison to normal dogs.

The increase in glucose pool after diabetes correlated with the increase in turnover rate (Figure 5.13). The relationship between glucose turnover rate and glucose pool was discussed earlier in the normal baboon. The marked increase in hepatic glucose production in the diabetic baboon resulted in a much greater glucose pool. If the increase in glucose pool is not balanced by increased peripheral utilization, as in the normal baboon, then glucose accumulation will be aggravated.

Glucose Space: The calculated glucose space decreased significantly from a mean of 8,8 litres in normal baboons to 6,8 litres after diabetes. Expressed as a fraction of body weight the mean glucose space was 31% before diabetes and 26% after diabetes. In previous studies on man glucose space (% body weight) in diabetes has been variously reported as increased (Shreeve *et al.* 1956), decreased (Hlad and Elrick, 1960), and unchanged (Forbath and Hetenyi, 1966; Manougian *et al.* 1964), in comparison to normal subjects. In dogs glucose space was reported to vary from 24 to 33% in several groups of normal dogs, and from 24 to 25% in pancreatectomised dogs (Ninomiya *et al.* 1965).

No meaningful comparison is possible between the results of previous studies and the present study. Widely different methods and techniques were used and diabetes was variable in type and severity in the different groups studied. The present study has an advantage in that comparison was not made between a normal group and a diabetic group but instead

each animal was its own control and therefore changes occurring after diabetes were more clearly defined. This is clearly illustrated in baboon B9 whose glucose space of 8,2 litres after diabetes was not significantly different from the mean glucose space of 8,8 litres in normal baboons. However, for baboon B9 this represented a 25% decrease in glucose space, as before becoming diabetic its glucose space was 11,4 litres.

The decrease in glucose space noted in this study must still be viewed with reservation as several other factors which affect the glucose space were not controlled. One such factor which must be emphasized is the level of hydration. A large fraction (if not most) of the estimated glucose space is extracellular fluid (Steele *et al.* 1956). Dehydration, which will be associated with a decreased extracellular fluid volume, will result in a significant reduction in the measured glucose space. Disturbances of hydration, particularly dehydration, are a common feature of severe diabetes and could have been partly responsible for the changes in glucose space noted in this study.

Of relevance is the fact that in studies on diabetic dogs the administration of insulin does not appear to produce a significant change in glucose space (Searle *et al.* 1954; Wrenshall and Hetenyi, 1959). This suggested that insulin deficiency by itself was unlikely to alter glucose space. In the present study there appeared to be a correlation between the decrease in glucose space and the degree of hyperglycaemia but, however, it was not statistically significant ( $r = + 0,63$ ;  $p < 0,10$ ). This tends to support the argument that the decrease in glucose space observed in the diabetic baboon was not simply a direct consequence of insulin deficiency. Thus it is likely that other factors, particularly dehydration, were relevant to the observed decrease in glucose space.

#### SUMMARY.

(1) Glucose turnover rates (total hepatic glucose production) vary widely in different species. In the normal baboon the mean turnover



rate was 904  $\mu\text{mol/kg/hr}$ , which is lower than the turnover rate reported in the dog but higher than that in man.

(2) The mean glucose space was 9 litres or 31% of body weight, and the glucose pool 1,5 mmol/kg. A significant positive correlation existed between the size of both the glucose pool and glucose space, and the glucose turnover rate.

(3) A significant increase in glucose turnover rates was a consistent finding after Streptozotocin Diabetes. The mean glucose turnover rate was 2709  $\mu\text{mol/kg/hr}$ , a threefold increase after diabetes. The increase in turnover rates was variable but correlated significantly with the degree of hyperglycaemia.

(4) The glucose pool increased nearly threefold after diabetes and correlated significantly with the increase in turnover rate. Glucose space decreased after diabetes, but the decrease did not correlate significantly with the degree of hyperglycaemia.

(5) The amount of glucose recycled increased after diabetes and correlated significantly with the glucose turnover rate. The fraction of the turnover rate recycled did not change significantly.

(6) A primary abnormality in carbohydrate metabolism in diabetes mellitus is hepatic overproduction of glucose resulting in a larger glucose pool and concomitant elevation in blood glucose concentration.

## CHAPTER SIX

## LACTATE KINETICS

Lactate turnover rates, pool and space were determined in ten normal baboons. The experiments were carried out usually two days after glucose kinetics studies had been completed. The housing, feeding and handling of the animals, prior to and on the day of the experiment have been described in detail in Chapter Three, page 20. Experiments were carried out between 8 a.m. and 12 noon, after a 14 to 16 hour fast. After a priming dose a constant rate infusion of  $[U-^{14}C]$  L-lactate was started. The experimental procedure, analytical procedures and calculations have been described in Chapter Three, pages 23-25.

After experiments were completed (including glucose kinetics and forearm studies) the baboons were made diabetic by an intravenous injection of streptozotocin, 50 mg/kg (Chapter Four). Lactate kinetics was then re-studied in the diabetic baboons, 48 hours after streptozotocin (before glucose kinetics was studied) in five baboons (B2, B3, B7, B8 and B9) and 96 hours after streptozotocin (after glucose kinetics was studied) in three baboons (B1, B4 and B6). Baboon B5 died as a result of streptozotocin toxicity, and B10 died 72 hours after producing diabetes. Thus lactate studies could not be repeated in both these baboons in the diabetic state.

The significance of changes in lactate kinetics after diabetes was analysed statistically by the *paired t-test*. Correlations were determined by the *Spearman rank* correlation coefficient.

RESULTS

All results, including those of previous studies, have been expressed in the International System of Units.

### Blood Lactate Specific Activities:

The blood lactate specific activity and the blood lactate concentration of representative experiments before and after diabetes are shown in Figure 6.1. Blood lactate concentrations remained relatively constant throughout the period of infusion, with slight fluctuations usually not exceeding 0,2 mmol/l. The fasting blood lactate concentration in the normal beboon was  $0,78 \pm 0,04$  (Mean  $\pm$  S.E.M.) and in the diabetic baboon  $1,74 \pm 0,14$ . The blood lactate specific activities reached a steady state (asymptote) after 105-120 minutes in the normal beboon, and after 90-100 minutes in the diabetic baboon. Fluctuations in the specific activity sometimes occurred after 150 minutes e.g. the 180 minute values in Figure 6.1, but these were usually less than 10 per cent. The mean asymptotic lactate specific activity in normal baboons ranged from 665 to 1550 dpm/ $\mu$ mol, and in the diabetic baboon from 428 to 741 dpm/ $\mu$ mol.

Significant negative correlations were found between the mean steady state lactate specific activity and the lactate pool and lactate turnover rate in the normal as well as in the diabetic baboon. As an example the relationships in the normal baboon are illustrated in Figures 6.2 and 6.3.

### Lactate Metabolism:

The Mean  $\pm$  S.E.M. of lactate turnover rates, blood lactate level, lactate pool and space, before and after diabetes are shown in figure 6.4 and 6.5.

The lactate turnover rates in ten normal and eight diabetic baboons are shown in Figure 6.6. In the case of baboons B5 and B10 only the normal lactate turnover rates are indicated. The lactate turnover rate in the normal baboon (mean blood glucose 4,75 mmol/l) was  $1128 \pm 38$   $\mu$ mol/kg/hr (Mean  $\pm$  S.E.M.). After the production of Streptozotocin Diabetes the lactate turnover rate in the diabetic baboon (mean blood glucose 18,56 mmol/l) was  $1927 \pm 133$   $\mu$ mol/kg/hr (Figure 6.4).

The increase in lactate turnover rates after the production of diabetes was statistically significant ( $p < 0,001$ ). Both before and after Streptozotocin Diabetes there was no significant correlation between lactate turnover rates and the blood lactate concentration. Significant positive correlations were found between the lactate turnover rate ( $\mu\text{mol/kg/hr}$ ) and the lactate pool ( $\mu\text{mol/kg}$ ), before and after diabetes. This relationship is illustrated in Figure 6.7.

#### Lactate Pool:

The Mean  $\pm$  S.E.M. of lactate pool, before and after diabetes, is shown in Figure 6.5.

The lactate pool in ten normal and eight diabetic baboons is shown in Figure 6.8. In the normal baboons the lactate pool was  $15,2 \pm 0,8$  mmol (Mean  $\pm$  S.E.M.). Expressed as a fraction of body weight the lactate pool was  $533 \pm 18$   $\mu\text{mol/kg}$  (Mean  $\pm$  S.E.M.). After Streptozotocin Diabetes the lactate pool was  $23,9 \pm 1,9$  mmol. Expressed as a fraction of body weight the lactate pool in the diabetic baboon was  $967 \pm 78$   $\mu\text{mol/kg}$ .

The increase in lactate pool after diabetes was statistically significant ( $p < 0,005$ ).

#### Lactate Space:

The Mean  $\pm$  S.E.M. of lactate space, before and after diabetes, is shown in Figure 6.5.

The lactate space in ten normal and eight diabetic baboons is shown in Figure 6.9. Lactate space in the normal baboon was  $19,2 \pm 1,3$  litres (Mean  $\pm$  S.E.M.). When expressed relative to body weight, the mean lactate space was 68% of body weight. After streptozotocin the lactate space was  $14,0 \pm 1,0$  litres in the diabetic baboon. The mean lactate space in the diabetic baboons represented 56% of body weight.

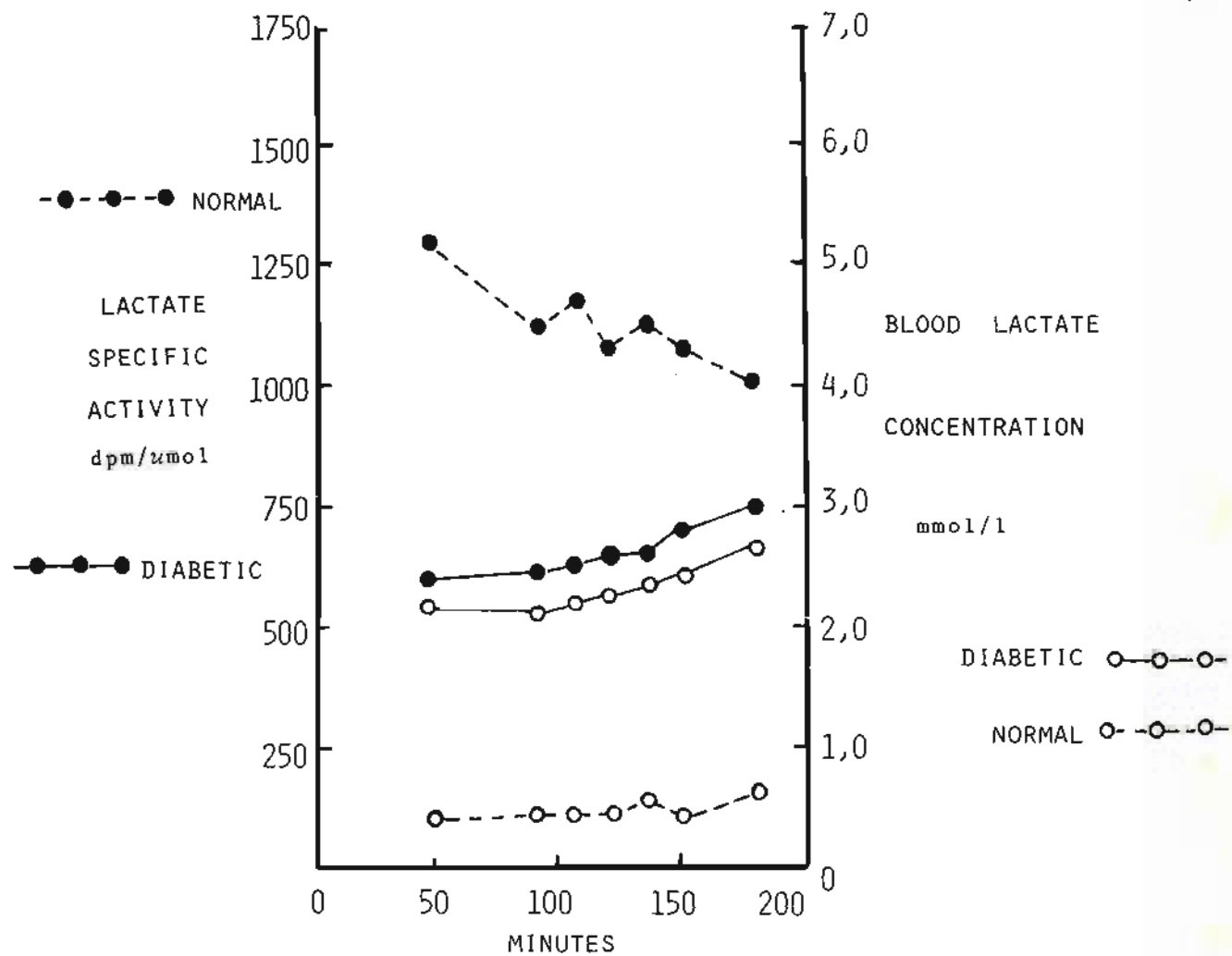


FIGURE 6.1 : BLOOD LACTATE CONCENTRATION AND LACTATE SPECIFIC ACTIVITY - TIME CURVES BEFORE AND AFTER DIABETES, IN BABOON B1.

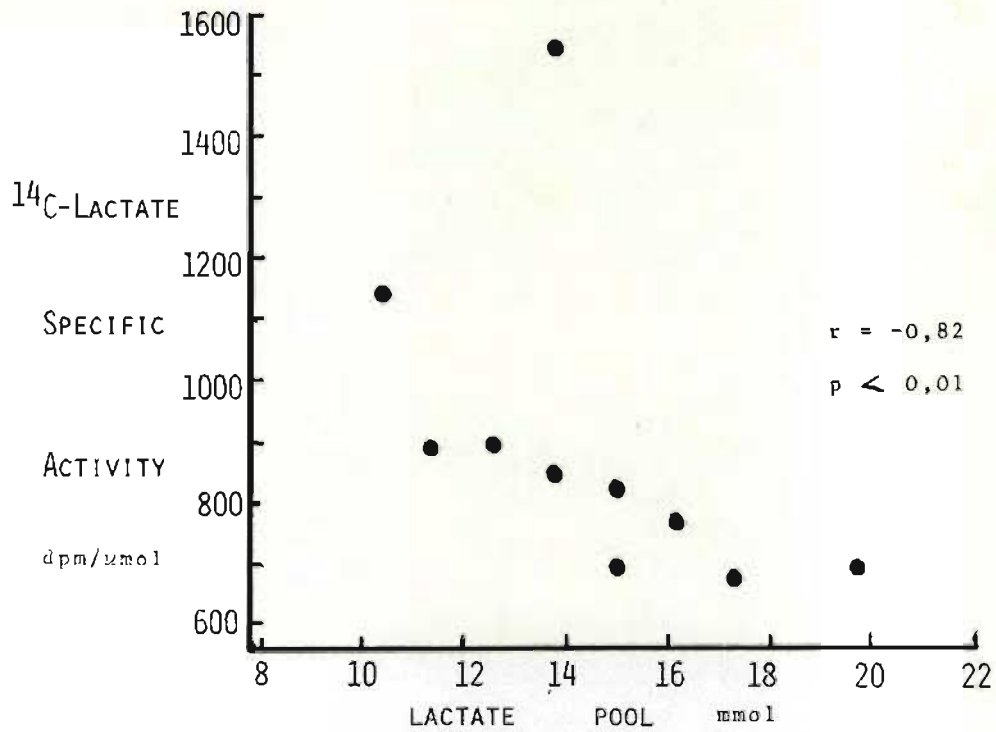


FIGURE 6.2 : CORRELATION BETWEEN STEADY STATE  $^{14}\text{C}$ -LACTATE SPECIFIC ACTIVITY AND LACTATE POOL IN THE NORMAL BABOON

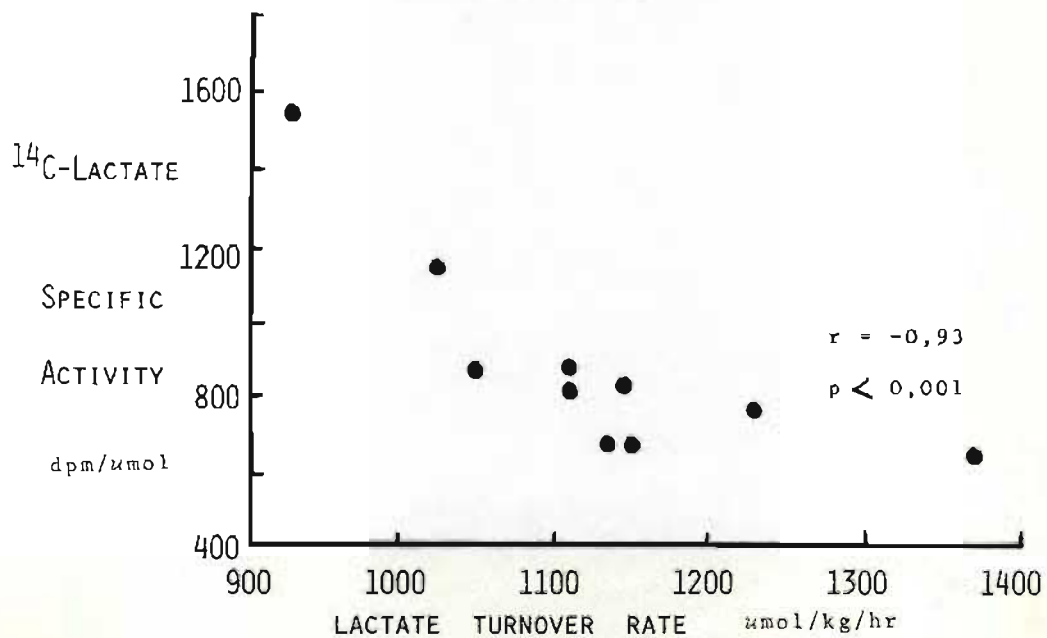


FIGURE 6.3 : CORRELATION BETWEEN STEADY STATE  $^{14}\text{C}$ -LACTATE SPECIFIC ACTIVITY AND LACTATE TURNOVER RATE IN THE NORMAL BABOON

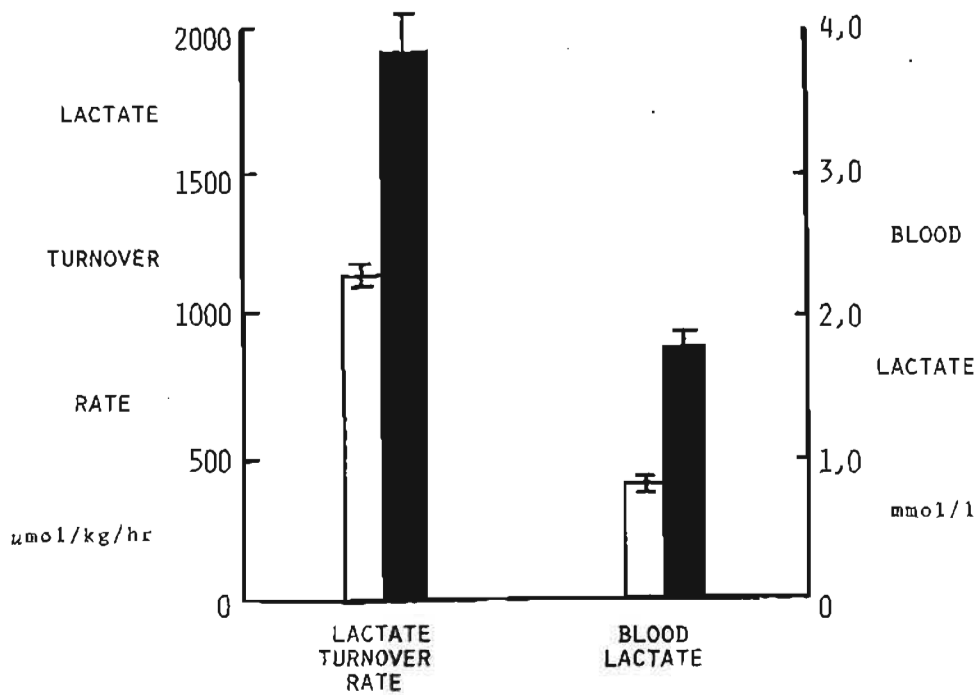


FIGURE 6.4 : LACTATE TURNOVER RATE AND BLOOD LACTATE CONCENTRATION. VALUES REPRESENT MEAN  $\pm$  S.E.M. BEFORE AND AFTER DIABETES.

BEFORE DIABETES
  AFTER DIABETES

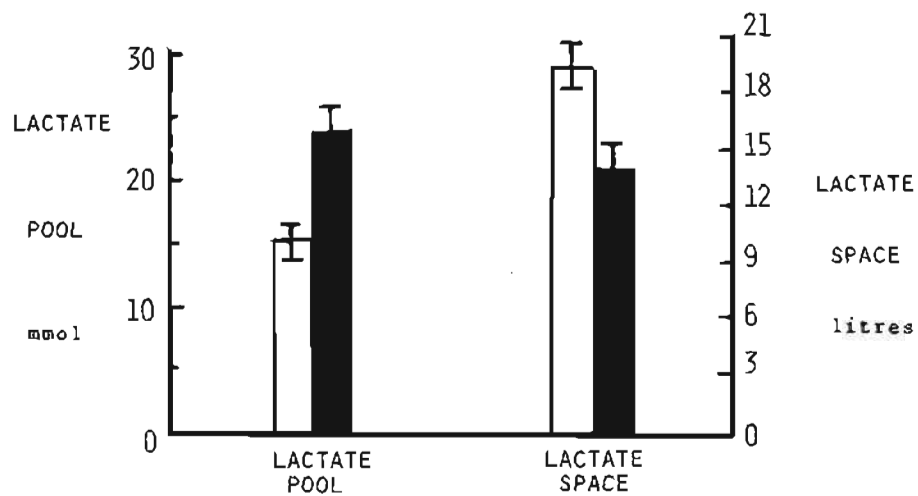


FIGURE 6.5 : LACTATE POOL AND LACTATE SPACE. VALUES REPRESENT MEAN  $\pm$  S.E.M. BEFORE AND AFTER DIABETES.

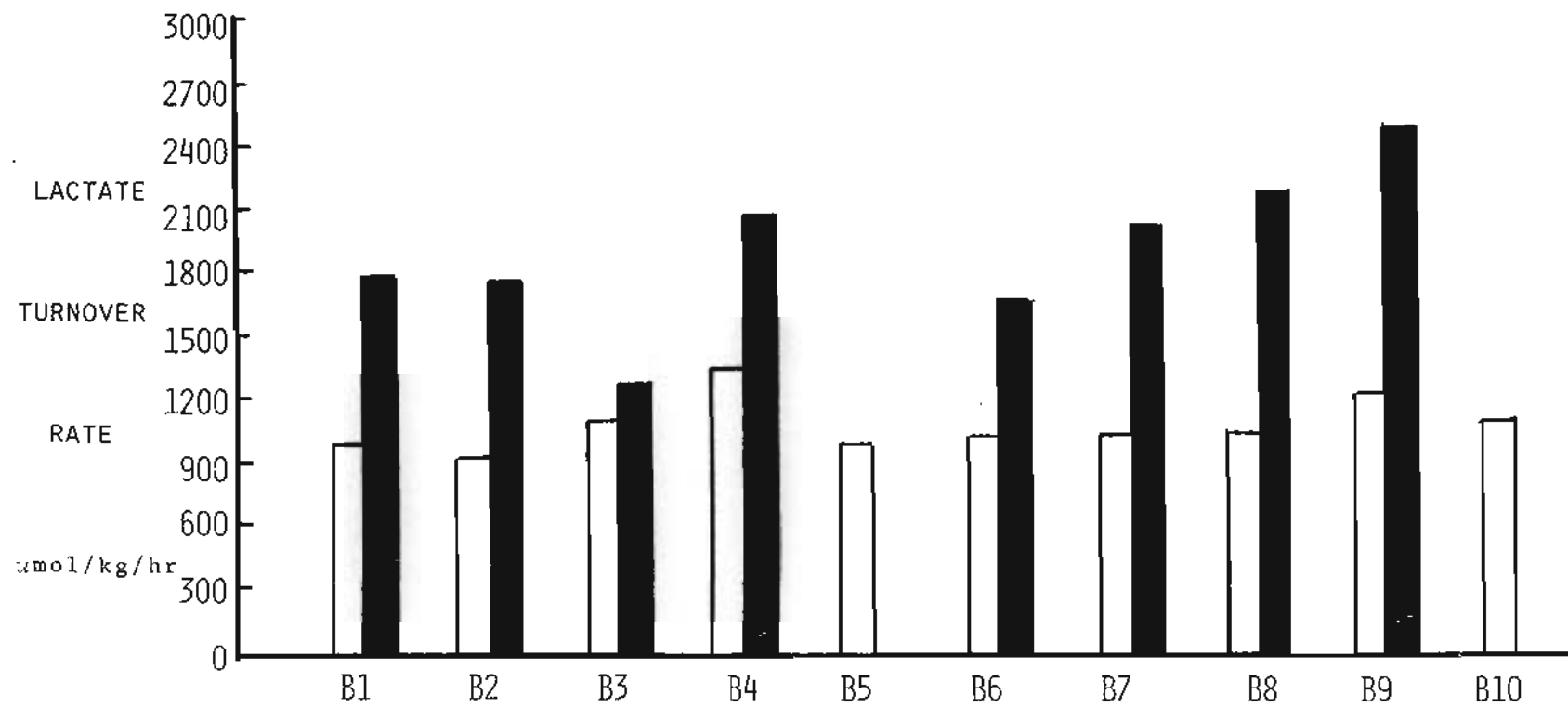



FIGURE 6.6 : LACTATE TURNOVER RATES BEFORE AND AFTER DIABETES.

NORMAL 

DIABETIC 



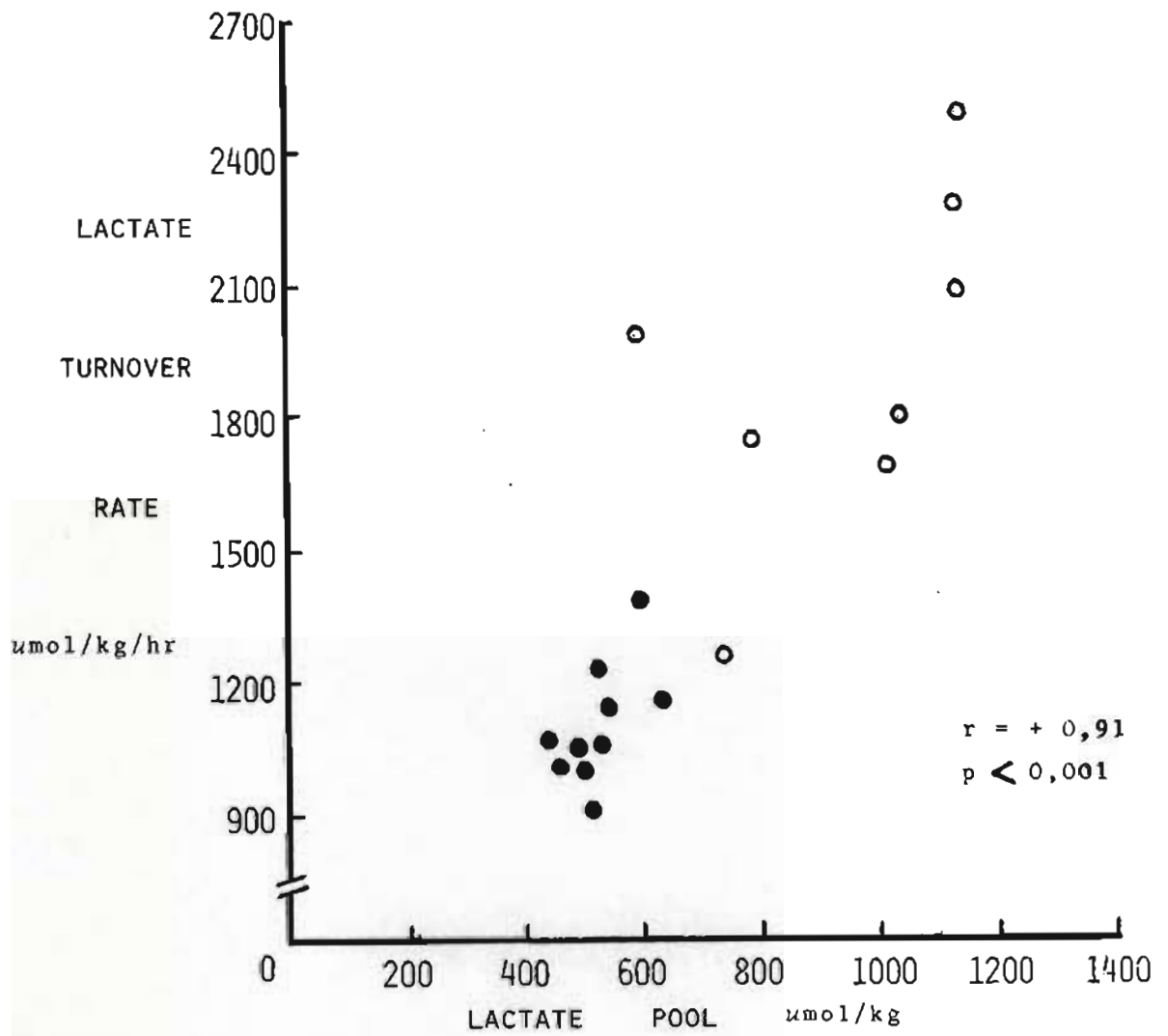


FIGURE 6.7 : CORRELATION BETWEEN LACTATE POOL AND LACTATE TURNOVER RATE IN THE NORMAL (●) AND DIABETIC (○) BABOON.

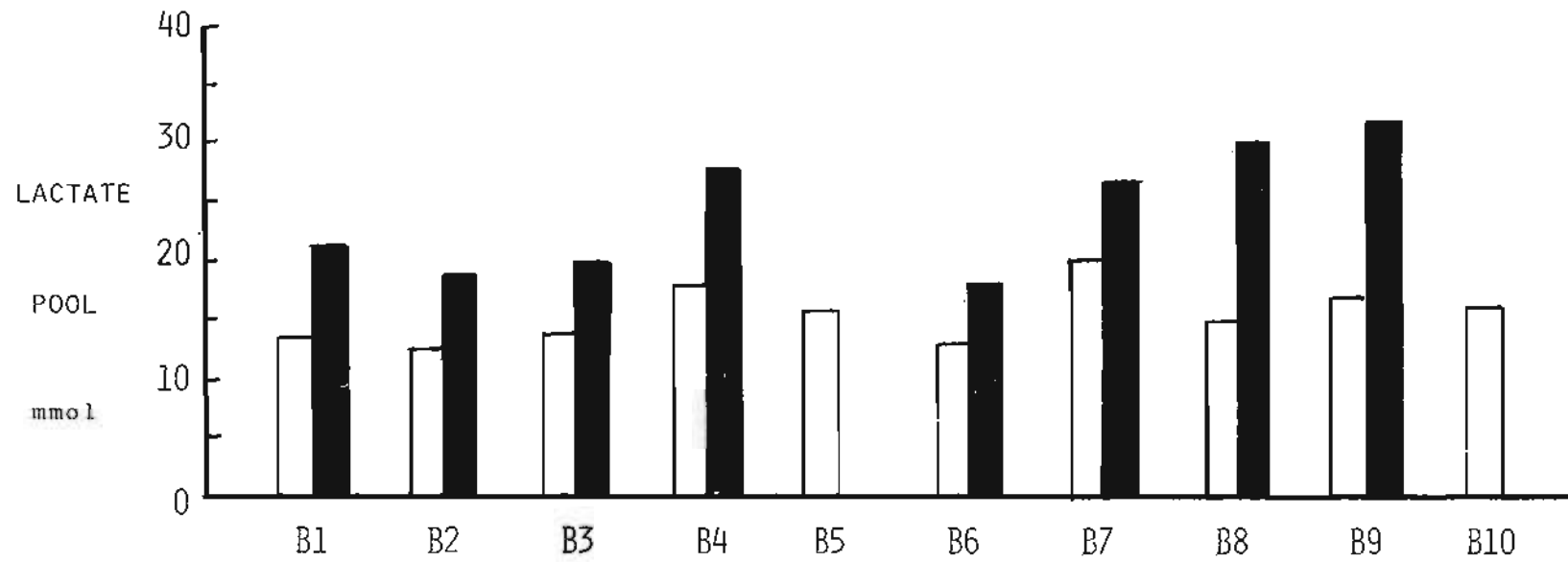


FIGURE 6.8 : LACTATE POOL BEFORE AND AFTER DIABETES.

NORMAL  DIABETIC

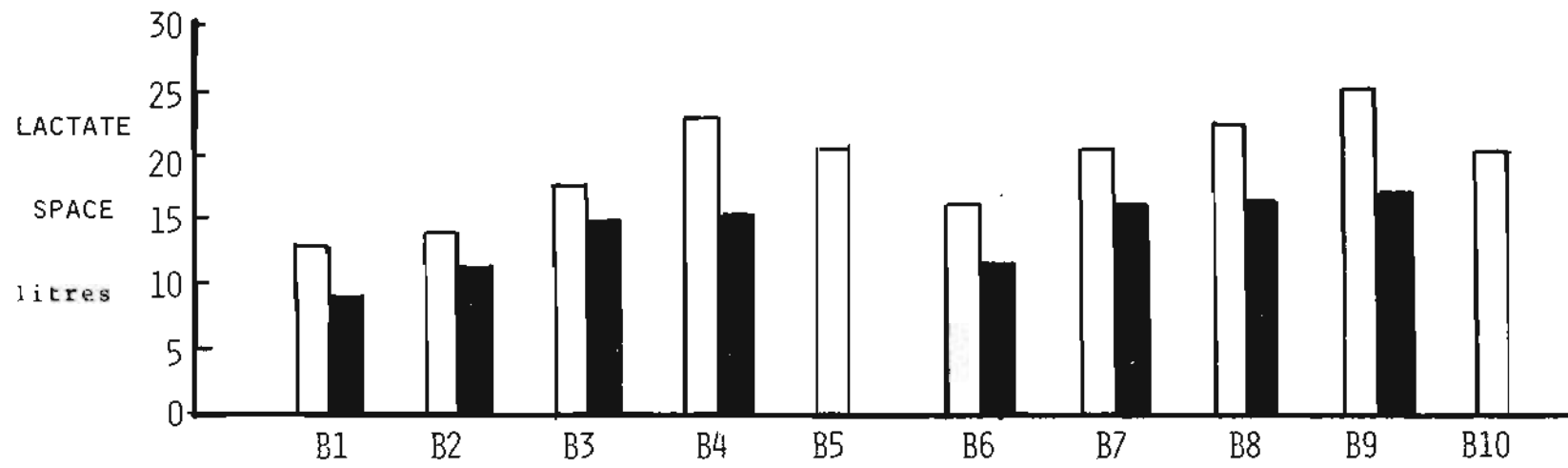


FIGURE 6.9 : LACTATE SPACE BEFORE AND AFTER DIABETES.

NORMAL       DIABETIC

The decrease in lactate space after diabetes was statistically significant ( $p < 0,001$ ).

### DISCUSSION

#### Lactate Kinetics in Normal Baboons:

The mean asymptotic lactate specific-activity varied considerably in the normal baboon. The highest value (1550 dpm/ $\mu$ mol) was more than twice the lowest value (665 dpm/ $\mu$ mol). All animals received identical amounts of [U- $^{14}$ C] L-lactate, both as a priming dose and thereafter as a constant rate infusion. Two of the factors which are important in determining the mean asymptotic specific activity are the lactate pool and the lactate turnover rate.

The lactate pool determines the extent of dilution of the labelled lactate. Thus the greater the lactate pool, the lower was the specific activity (Figure 6.2). The lactate turnover rate reflects the rate at which endogenous lactate, and hence the labelled lactate, is being metabolised. The higher the lactate turnover rate, the greater the rate of utilization of the infused labelled lactate and hence the lower the asymptotic specific activity (Figure 6.3). In addition, higher turnover rates (and therefore higher endogenous production rates) would result in a larger lactate pool (Figure 6.7) and hence a lower specific activity, as mentioned above.

The mean lactate turnover rate in ten normal baboons was 1128  $\mu$ mol/kg/hr. There was considerable individual variation (928 to 1384  $\mu$ mol/kg/hr), the highest turnover rate being 1,5 times the lowest. The degree of individual variation was, however, less than that observed with glucose turnover rates. The significant positive correlation (Figure 6.7) between the lactate turnover rate ( $\mu$ mol/kg/hr) and lactate pool ( $\mu$ mol/kg) indicates two significant relationships. Firstly, the rate of production of lactate determines the lactate pool, and secondly, the

rate of utilization of lactate is determined by the lactate pool. Thus changes in the rate of production of lactate alter the size of the lactate pool resulting in corresponding changes in the rate of lactate utilization.

There are, to the author's knowledge, no reports of lactate turnover rates in baboons. The only possible comparison is between the results in the baboon and those reported in other species including man. Table 6.1 below shows normal lactate turnover rates reported in other species. In all these studies lactate turnover rates were determined during a primed infusion of  $[U-^{14}C]$  L-lactate after an overnight fast.

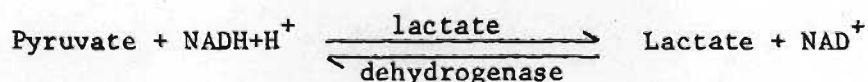
**TABLE 6.1** NORMAL LACTATE TURNOVER RATES IN THE RAT, DOG, BABOON AND MAN.

Reference	Species	No. Studied	Mean Turnover Rate $\mu\text{mol/kg/hr}$
KREISBERG <i>et al.</i> 1970	man	5	904
SEARLE and CAVALIERI, 1972	man	4	1072
FORBATH <i>et al.</i> 1967	dog	7	1434
WIENER and SPITZER, 1974	dog	6	2154
FREMINET <i>et al.</i> 1974	rat	10	4000
Present study	baboon	10	1128

The results in Table 6.1 show that lactate turnover rates are highest in the rat and lowest in man, while the turnover rate in the dog is intermediate. The mean lactate turnover rate in the baboon is higher than in man but lower than in the dog. Thus lactate turnover rates determined in the baboon fall within the range of values reported in other species. The variation in lactate turnover rate amongst the different species is similar to that observed with glucose turnover rates.

As outlined in the introductory chapter (Chapter One) [ $U-^{14}C$ ] L-lactate is also recycled via glucose. The data obtained in the present study does not allow an estimate of recycling and hence the degree of underestimation of the lactate turnover rate. In the experiments of Kreisberg *et al.* (1971), on normal subjects, 21% of the lactate turnover was converted to glucose and 45% of the glucose turnover was converted to lactate. From these results a theoretical estimate of lactate recycling would be about 9,5% (i.e. 45% of 21%). However, the extent of recycling has not been determined experimentally. This can be achieved by using a lactate tracer which loses its "label" irreversibly during an early stage in the metabolism of lactate. The use of a tritiated lactate tracer, such as [ $2-^3H$ ] L-lactate is worthy of investigation as the labelled hydrogen would be lost in the conversion of lactate to pyruvate, the first and obligatory step in the metabolism of lactate. Thus recycling of the "label" would be attenuated and a more accurate estimation of lactate turnover rate made. Unfortunately, at the time the present study was undertaken tritiated lactate was not available commercially.

The mean lactate pool in the normal baboon was 15,2 mmol or 533  $\mu$ mol/kg body weight. Variation in the lactate pool was present. Lactate is produced from pyruvate, and several factors influence its rate of production and consequently the size of the lactate pool.



These factors include

- (i) the rate of production of pyruvate (i.e. the rate of glycolysis);
- (ii) the rate of utilization of pyruvate (which depends on the activity of the mitochondrial enzyme pyruvate dehydrogenase and the availability of oxygen at the cellular level); and
- (iii) the redox state of the cell (i.e. the ratio of NADH to  $\text{NAD}^+$ ).

Apart from inherent differences, the influence of these factors is also modified in a highly variable manner by the markedly different type and amount of spontaneous muscular activity in each animal prior to sedation. Thus a high level of spontaneous exercise would result in increased levels of pyruvate and NADH (because of increased anaerobic glycolysis) and consequently increased formation of lactate.

There are few reports of lactate pool estimation in other species. The lactate pool in four normal subjects was reported to range from 256 to 389  $\mu\text{mol/kg}$  with a mean of 322  $\mu\text{mol/kg}$  (Searle and Cavalieri, 1972). This is just over half the estimated lactate pool in the baboon. On the assumption that lactate is distributed throughout body water, lactate pool was estimated to be 400  $\mu\text{mol/kg}$  in human subjects (Kreisberg *et al.* 1970) and 333 to 556  $\mu\text{mol/kg}$  in the dog (Forbath and Hetenyi, 1970). In a recent study, using the single injection technique, lactate pool in the normal dog was reported to be 522 to 556  $\mu\text{mol/kg}$  (Belo *et al.* 1977). Lactate pool in the baboon thus appears to be similar to that reported in the dog but higher than in man.

Lactate space in the normal baboon ranged from 12,8 to 25,3 litres with a mean of 19,2 litres. Expressed as a fraction of body weight the mean lactate space was 68%. In a study on four normal subjects (Searle and Cavalieri, 1972) the lactate space was reported to vary from 43 to 57% of body weight with a mean of 49,4%. The estimated lactate space in the baboon is thus nearly 40% greater than reported for man.

There are no other reports of lactate space estimation in man or other species with which to compare the results of Searle and Cavalieri (1972) in man, and that of the present study in the baboon. Lactate is a freely and rapidly diffusible molecule and is therefore assumed to be distributed throughout the body water (Kreisberg *et al.* 1970; Belo *et al.* 1977). Lactate space should therefore be equivalent to the total body water space. The value for total body water space (as a fraction of body weight) in the normal baboon is not known, but it is approximately 69% in the adult monkey and 60% in the dog (Altman and

Dittmer, 1974). The value in the baboon is likely to be in the region of 60 to 70%. It is therefore particularly reassuring to note that the estimated lactate space in the normal baboon in the present study (68%) is in reasonable agreement with its likely body water space.

Although the lactate pool is small relative to the glucose pool, the turnover rate of lactate is significantly greater than the glucose turnover rate. Thus possible errors in the estimation of lactate turnover rate, pool and space, due to recycling and isotopic exchange would be greater than in the case of glucose. The actual values obtained should be interpreted with caution and instead more emphasis placed on the overall pattern, both in the normal as well as in the diabetic animal.

#### Lactate Kinetics in Diabetic Baboons:

The mean steady state specific activities in the diabetic baboons were about 35 to 40% lower than during similar experiments before diabetes. This was because of the increased lactate turnover rates and lactate pool after diabetes. The labelled lactate was thus metabolised (and hence removed) at a faster rate and also the increased lactate pool resulted in dilution of the infused labelled lactate to a greater extent. For these reasons the mean asymptotic specific activity was significantly lower in the diabetic baboon despite it receiving the same amount of [U-<sup>14</sup>C] L-lactate as in the experiment before diabetes.

Lactate turnover rates were increased significantly in all baboons after the production of Streptozotocin Diabetes. The mean turnover rate of 1927  $\mu\text{mol/kg/hr}$  was more than 70% higher than the mean lactate turnover rate in the normal baboon.

In an early study, Forbath *et al.* (1967) used both the single injection and primed infusion tracer techniques to determine lactate turnover rates in seven normal and seven pancreatectomized dogs. Using a primed infusion of [U-<sup>14</sup>C] L-lactate, the mean lactate turnover



## CHAPTER SEVEN

## FOREARM METABOLISM

The forearm technique was used to study the peripheral metabolism of glucose, lactate, triglycerides, free fatty acids and glycerol. Peripheral metabolism was investigated at the same time as glucose turnover studies were carried out. Details of the technique and experimental procedure have been described in Chapter Three, pages 25-27.

Ten normal baboons were studied and nine after diabetes. In four baboons (B1, B4, B6 and B10) the investigations were made 48 hours after streptozotocin, and in the remaining five (B2, B3, B7, B8 and B9) 96 hours after the drug.

The concentration of glucose and lactate in blood, and triglyceride, free fatty acid and glycerol in plasma, were determined by the methods described in Appendices One and Two, and Five to Seven. Results were analysed statistically by using the *paired t-test* (thus the significance of changes after diabetes were determined relative to the normal values for each individual baboon). Correlations were determined by the *Spearman rank* correlation coefficient.

The assessment of peripheral metabolism was made just prior to commencing the glucose turnover study and then repeated after one hour, during the turnover study.

## RESULTS

Results of the two separate assessments of forearm metabolism were not

significantly different. Accordingly, the results presented are combined data from both experiments and are expressed as Mean  $\pm$  S.E.M.

Forearm Blood Flow: Forearm blood flow (the mean of four estimations) in each baboon, before and after streptozotocin, is illustrated in Figure 7.1. In the normal baboon mean forearm blood flow was  $5,1 \pm 0,28$  ml/100ml/min, and after streptozotocin it increased significantly ( $p < 0,001$ ) to  $9,9 \pm 0,59$  ml/100ml/min. No significant correlation was found between the increase in forearm blood flow and the degree of hyperglycaemia.

The arterial levels and nett forearm exchange of all substrates studied, before and after diabetes, are shown in Figure 7.2.

Glucose: The arterial glucose concentration and nett forearm exchange of glucose in each baboon, before and after streptozotocin, are shown in Figure 7.3.

The mean arterial glucose concentration in the normal baboon was  $5,03 \pm 0,15$  mmol/l, and after streptozotocin it increased significantly ( $p < 0,001$ ) to  $20,6 \pm 1,66$  mmol/l (Figure 7.2).

Nett uptake of glucose was observed in the forearm of the normal baboon. The mean glucose uptake was  $1,95 \pm 0,25$   $\mu$ mol/100g/min (Figure 7.2). After the production of diabetes glucose uptake was observed in only two baboons, B2 and B7, but was reduced by 51% and 41% respectively. In the remaining seven baboons nett production of glucose was observed (Figure 7.3). Thus overall forearm exchange of glucose in the diabetic baboon consisted of nett release of  $1,65 \pm 0,63$   $\mu$ mol/100g/min (Figure 7.2). The decreased uptake and nett release of glucose after Streptozotocin Diabetes was statistically significant ( $p < 0,001$ ).

A significant positive correlation was found between the arterial glucose concentration and forearm glucose uptake in the normal baboon (Figure 7.4).

After diabetes a significant inverse correlation was found between glucose level and glucose uptake (Figure 7.5).

Lactate: The arterial lactate concentration and nett forearm exchange of lactate in each baboon, before and after diabetes, is illustrated in Figure 7.6.

The mean arterial lactate concentration in the normal baboon was  $0,73 \pm 0,06$  mmol/l and after the production of diabetes it increased significantly ( $p < 0,001$ ) to  $2,30 \pm 0,41$  mmol/l (Figure 7.2).

In both the normal and diabetic baboons there was nett release of lactate in the forearm. Mean lactate release in the normal baboon was  $0,55 \pm 0,1$   $\mu$ mol/100g/min and increased significantly ( $p < 0,001$ ) after diabetes to  $2,22 \pm 0,27$   $\mu$ mol/100g/min (Figure 7.2).

A significant positive correlation was found between forearm lactate release and glucose uptake in the normal baboon (Figure 7.7). This relationship was not observed in the diabetic baboon.

Triglyceride: Figure 7.8 illustrates the arterial triglyceride concentration and nett forearm exchange of triglycerides in each baboon, before and after diabetes.

The mean arterial triglyceride concentration in the normal baboon was  $0,71 \pm 0,05$  mmol/l, and after the production of diabetes it increased significantly ( $p < 0,001$ ) to  $2,42 \pm 0,33$  mmol/l. Triglyceride concentrations were increased in all baboons except B2 in which there was no significant change.

A significant correlation was noted between arterial triglyceride and free fatty acid concentrations in the normal baboon (Figure 7.9). A similar relationship was observed in the diabetic baboon but it was not statistically significant ( $p < 0,1 > 0,05$ ).

In the normal baboon there was a nett uptake of triglyceride in the forearm. Mean uptake was  $1,33 \pm 0,2 \mu\text{mol}/100\text{g}/\text{min}$  (Figure 7.2). Following the induction of diabetes the pattern of triglyceride exchange was variable: in baboon B2 and baboon B9 there was decreased uptake and in the remaining seven baboons nett triglyceride release was recorded (Figure 7.8). Overall, the mean forearm exchange of triglyceride in the diabetic baboon was a nett release of  $1,8 \pm 0,50 \mu\text{mol}/100\text{g}/\text{min}$  (Figure 7.2). The altered pattern of triglyceride exchange in the forearm after diabetes was statistically significant ( $p < 0,001$ ).

A significant positive correlation was found between glucose and triglyceride uptake in the forearm of the normal baboon (Figure 7.10). This was not observed in the diabetic baboon.

Free Fatty Acid (FFA): Figure 7.11 illustrates the arterial FFA concentration and nett forearm exchange of FFA in each baboon, before and after diabetes.

The mean arterial FFA concentration in the normal baboon was  $0,37 \pm 0,05 \text{ mmol}/\text{l}$ , and after streptozotocin it increased significantly ( $p < 0,001$ ) to  $0,97 \pm 0,11$  (Figure 7.2). An increase in FFA concentration occurred in all baboons except B2.

Forearm FFA exchange was variable. In two normal baboons there was a small ( $0,03$ - $0,10 \mu\text{mol}/100\text{g}/\text{min}$ ) nett release of FFA. In the remaining eight baboons there was a nett uptake of FFA (Figure 7.11). Thus, overall, there was a mean uptake of  $0,13 \pm 0,05 \mu\text{mol}/100\text{g}/\text{min}$  (Figure 7.2). After streptozotocin a nett release of FFA was observed in all baboons except B2 (Figure 7.11). In B2 nett uptake occurred but was reduced to 50% of its normal value. Overall mean exchange after diabetes was a nett release of  $0,40 \pm 0,17 \mu\text{mol}/100\text{g}/\text{min}$  (Figure 7.2).

The release of FFA after diabetes was statistically significant ( $p < 0,01$ ). A significant negative correlation was found between the arterial lactate

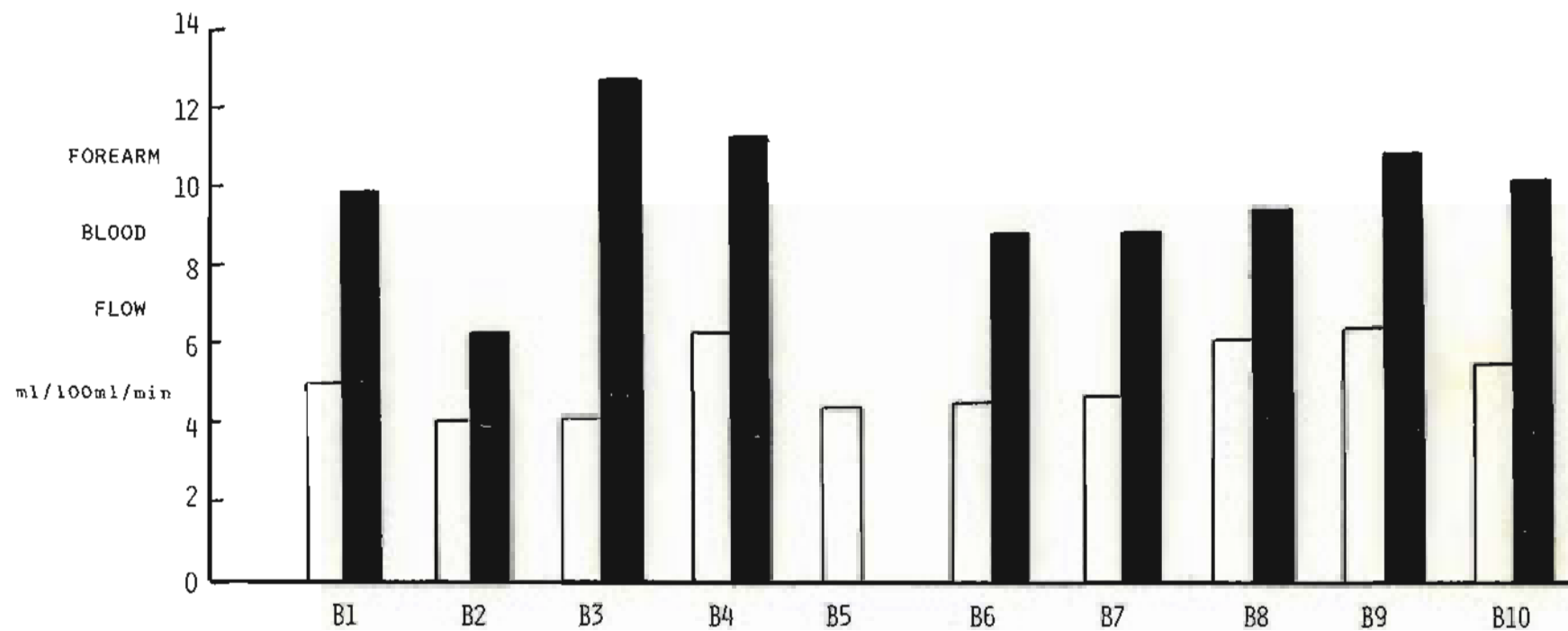


FIGURE 7.1 : FOREARM BLOOD FLOW BEFORE AND AFTER DIABETES.

NORMAL  DIABETIC

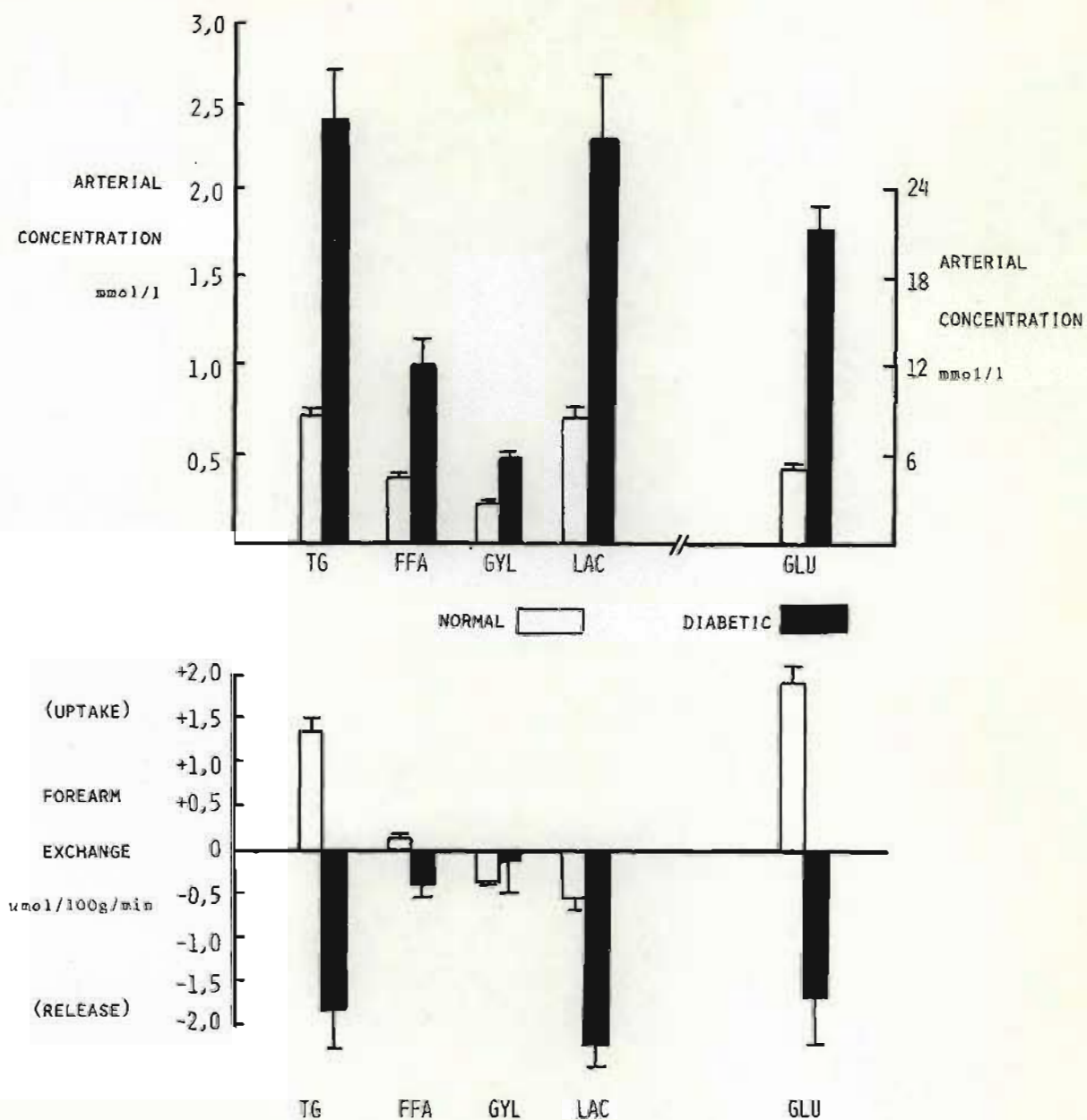


FIGURE 7.2 : ARTERIAL CONCENTRATIONS AND FOREARM EXCHANGE OF TRIGLYCERIDE (TG), FREE FATTY ACIDS (FFA), GLYCEROL (GYL), LACTATE (LAC), AND GLUCOSE (GLU). VALUES ARE MEAN  $\pm$  S.E.M.

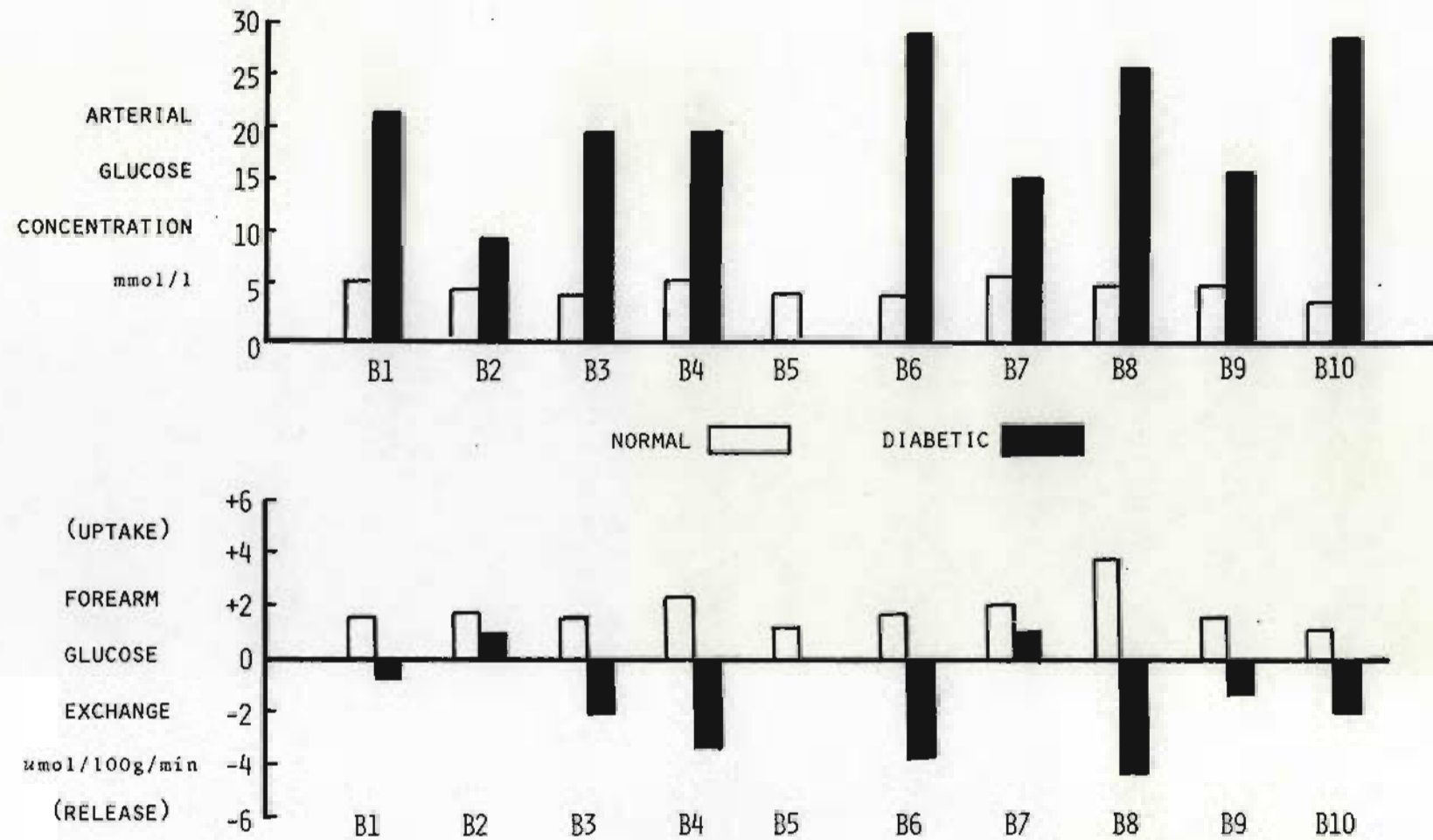


FIGURE 7.3 : ARTERIAL CONCENTRATIONS AND FOREARM EXCHANGE OF GLUCOSE BEFORE AND AFTER DIABETES.

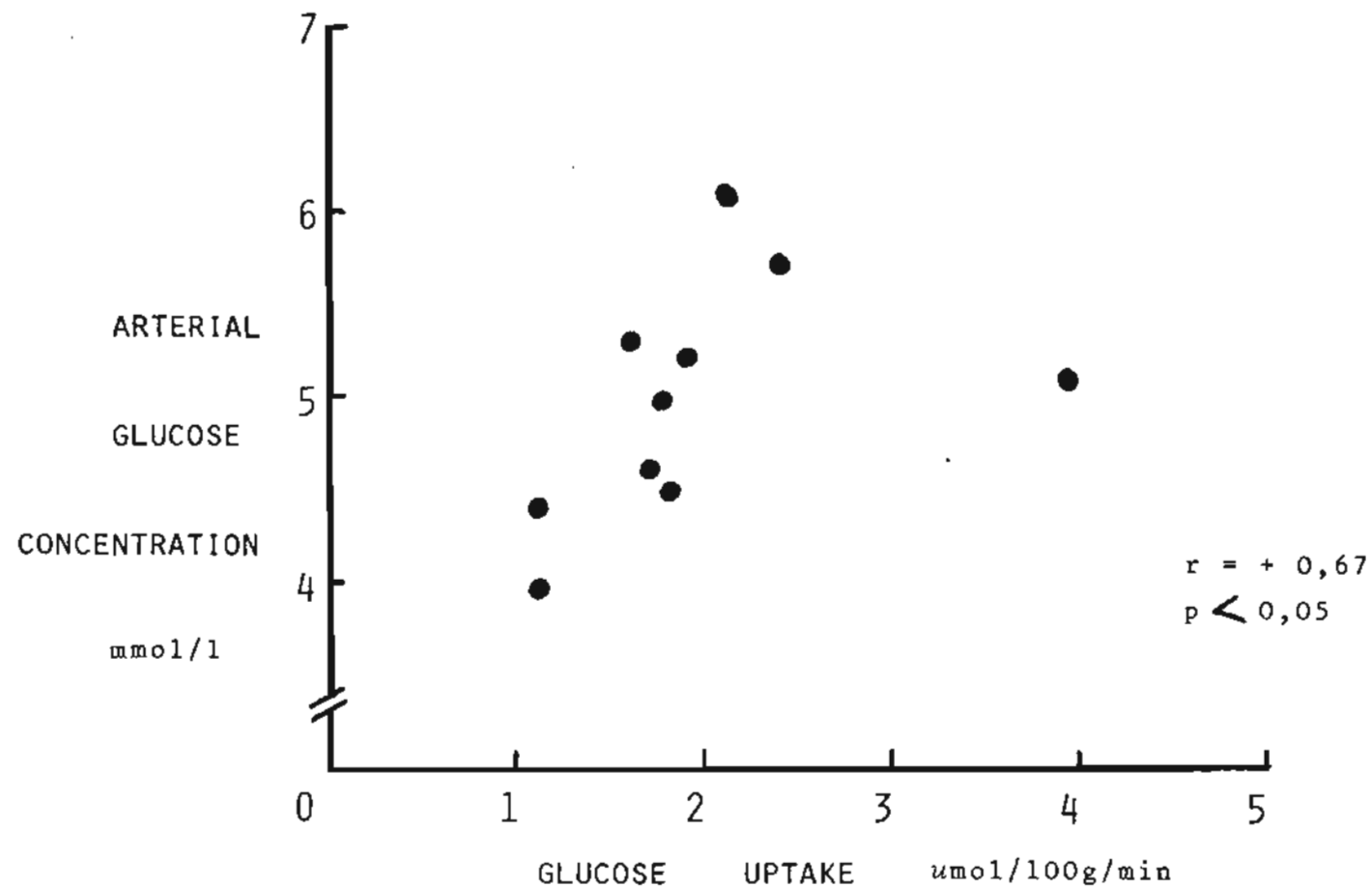


FIGURE 7,4 : CORRELATION BETWEEN ARTERIAL GLUCOSE CONCENTRATION AND GLUCOSE UPTAKE IN THE NORMAL BABOON.



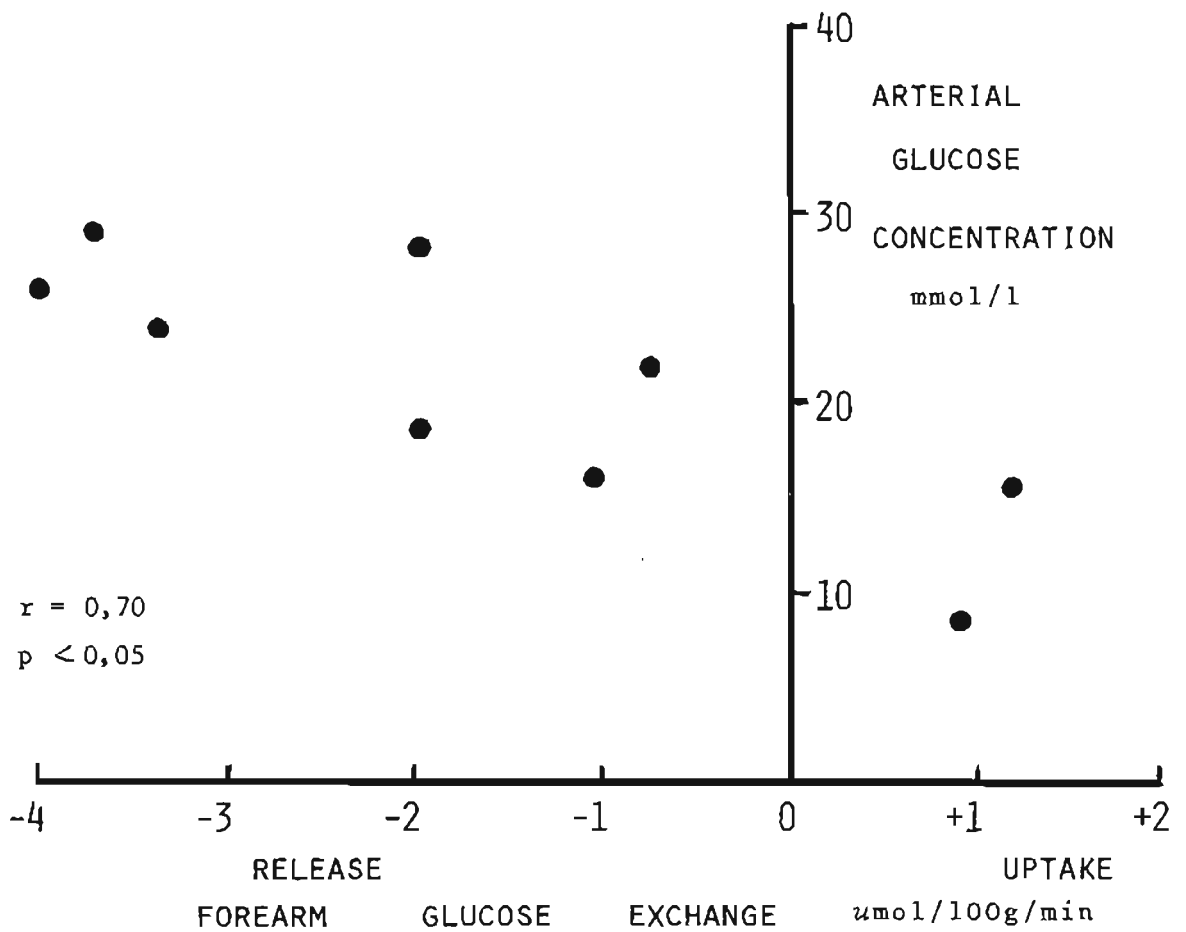


FIGURE 7,5 : CORRELATION BETWEEN ARTERIAL CONCENTRATION AND FOREARM EXCHANGE OF GLUCOSE IN THE DIABETIC BABOON.

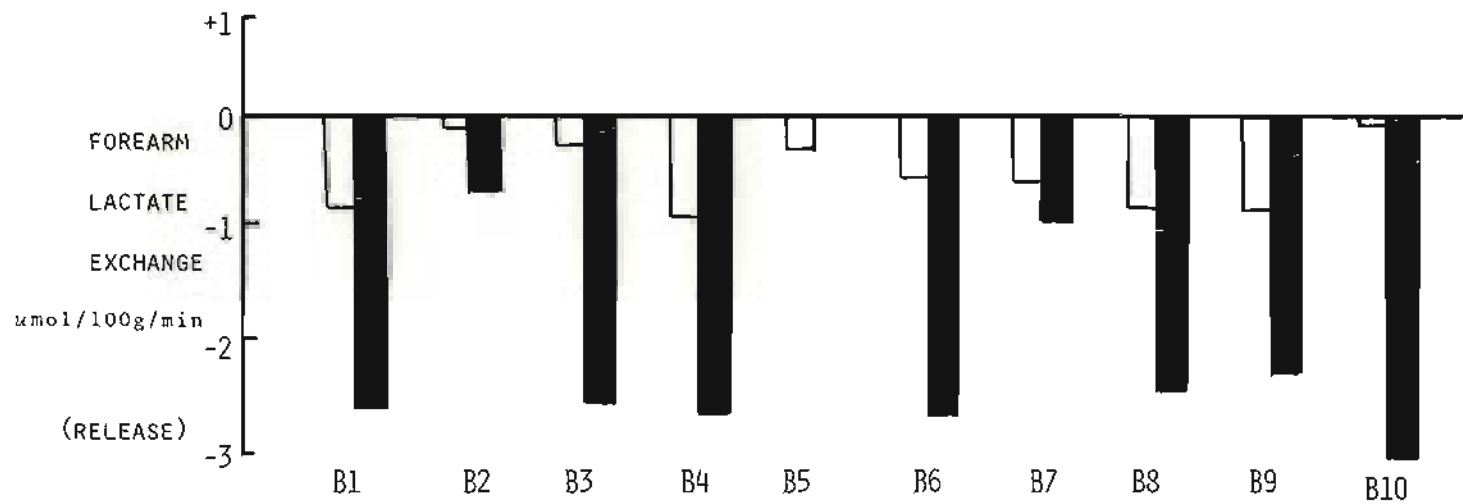
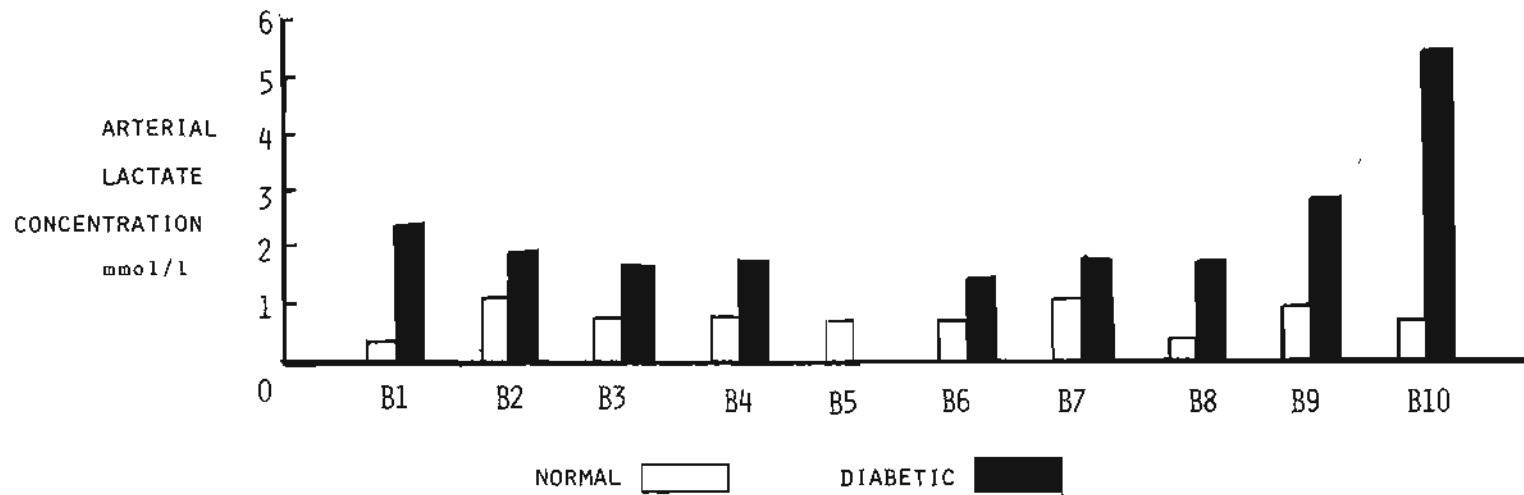


FIGURE 7.6 : ARTERIAL CONCENTRATIONS AND FOREARM EXCHANGE OF LACTATE BEFORE AND AFTER DIABETES.

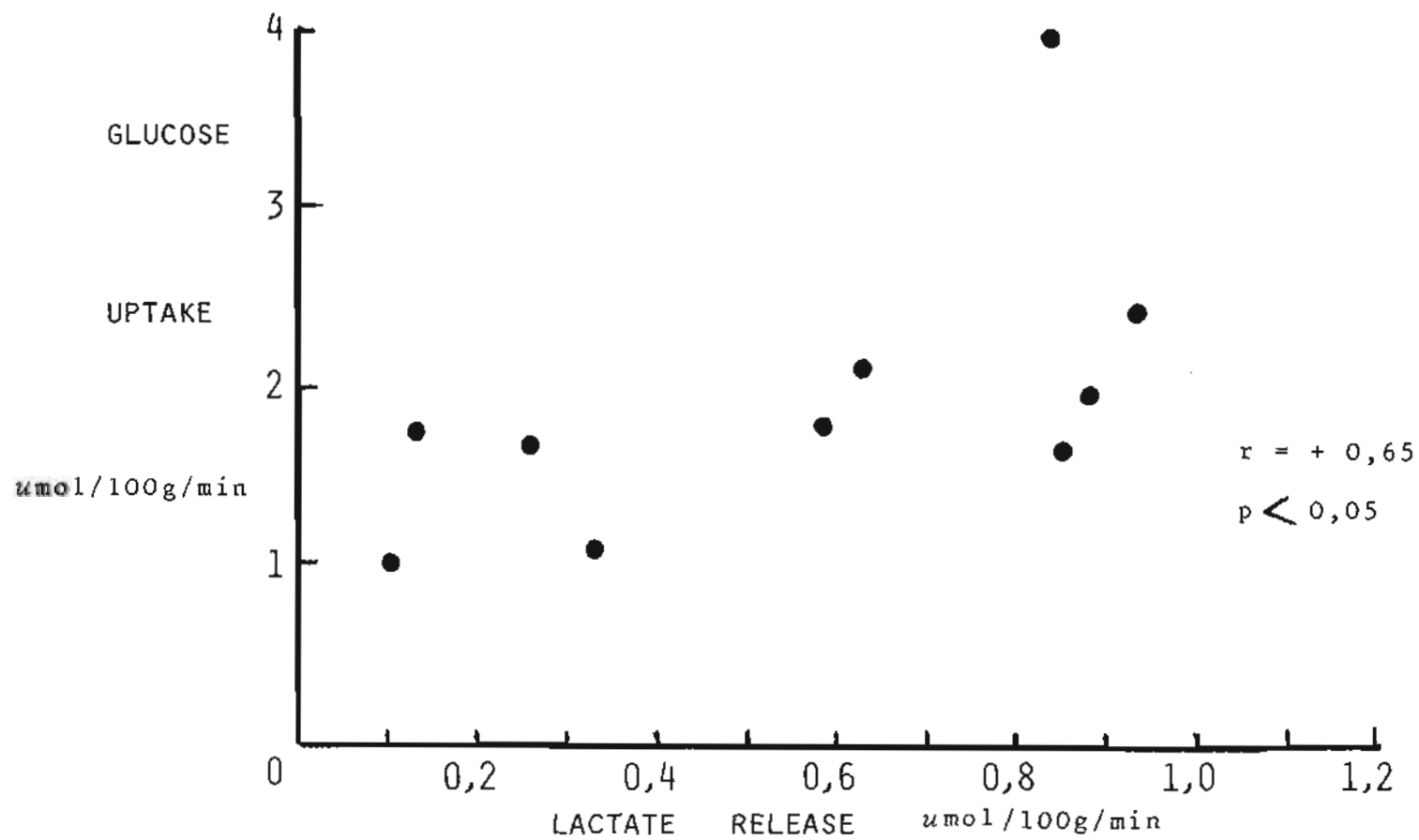


FIGURE 7.7 : CORRELATION BETWEEN GLUCOSE UPTAKE AND LACTATE RELEASE IN THE NORMAL BABOON.

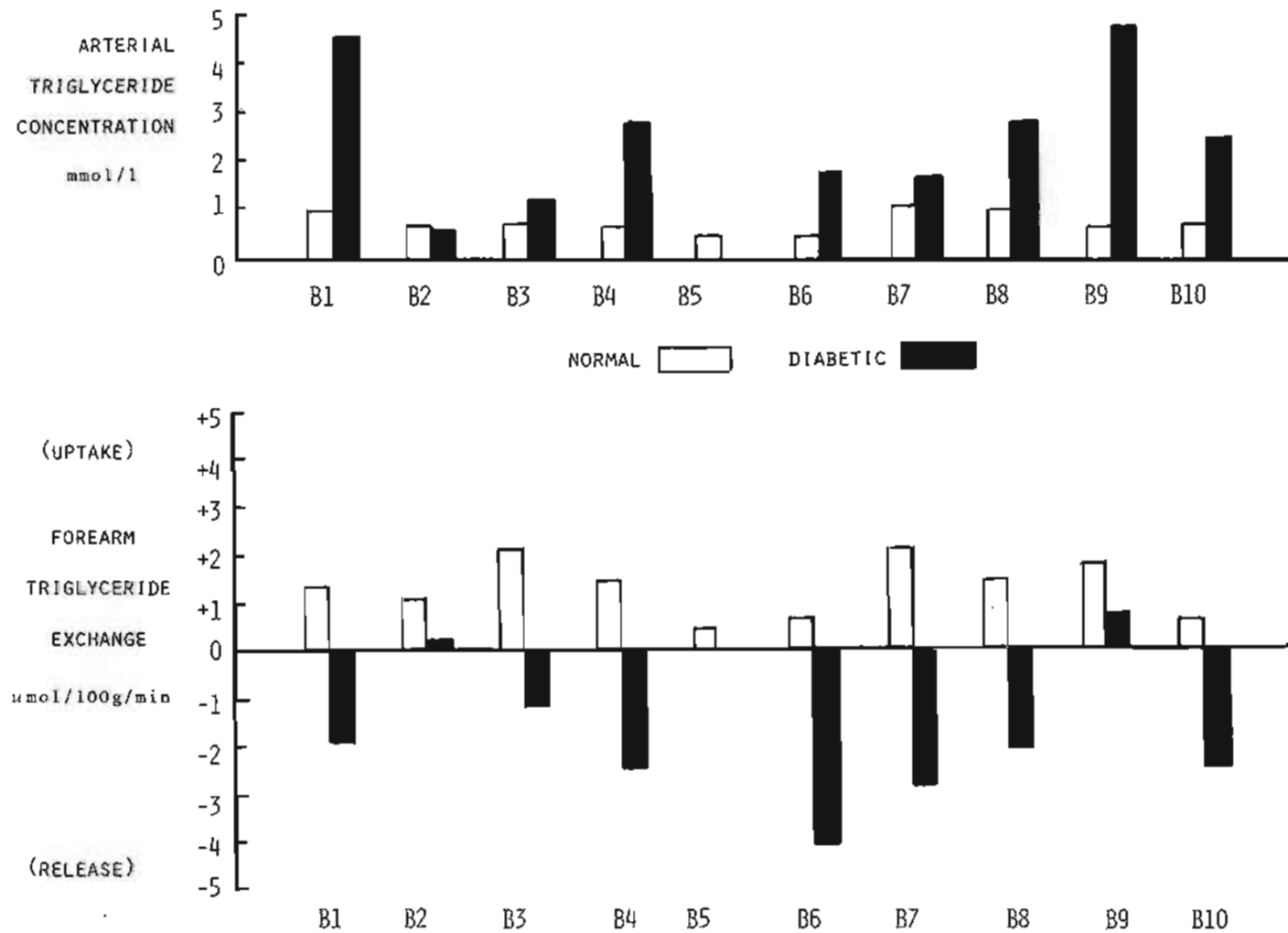


FIGURE 7.8 : ARTERIAL CONCENTRATIONS AND FOREARM EXCHANGE OF TRIGLYCERIDE BEFORE AND AFTER DIABETES.

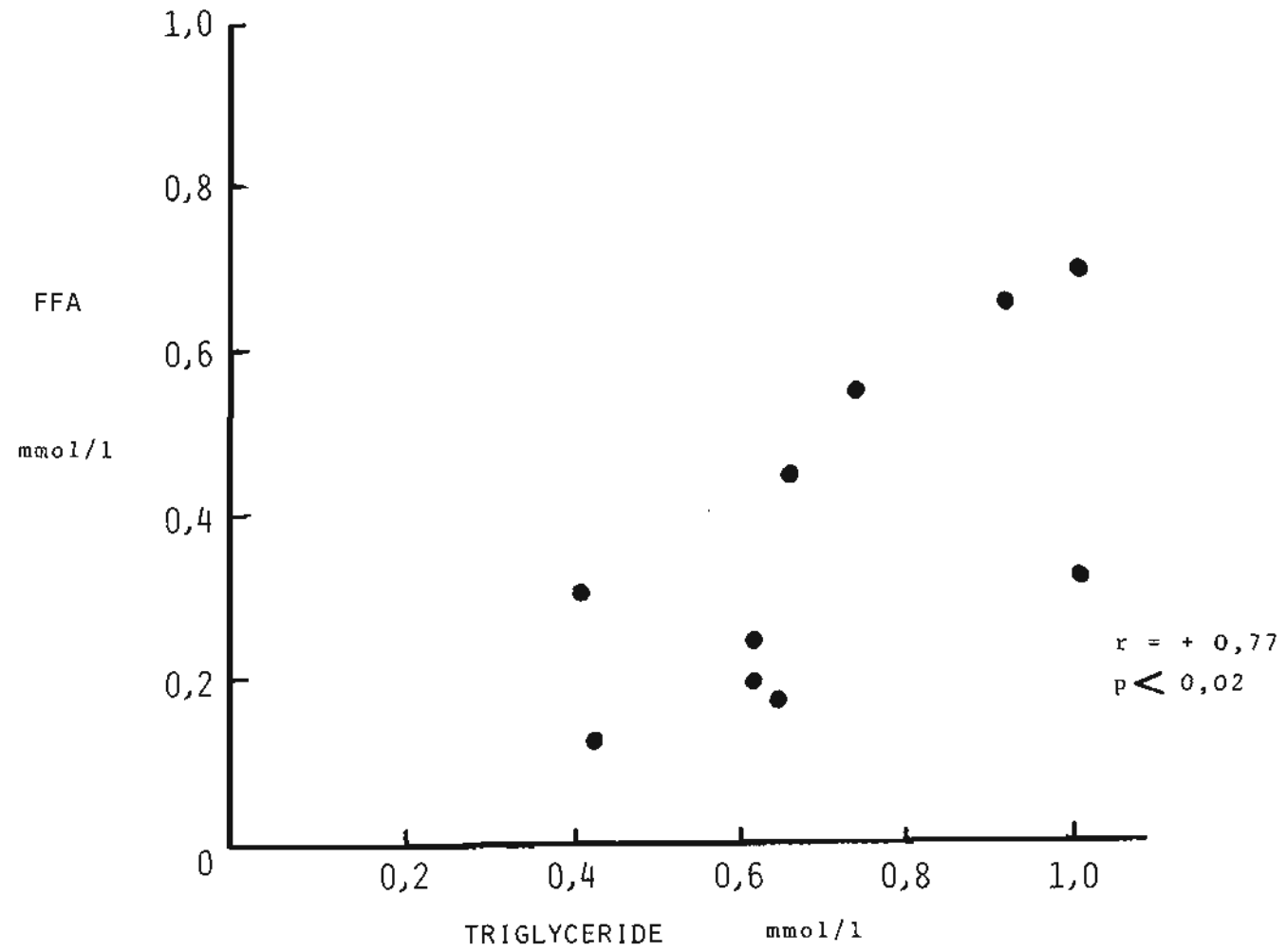


FIGURE 7.9 : CORRELATION BETWEEN ARTERIAL FFA AND TRIGLYCERIDE LEVELS  
IN THE NORMAL BABOON.

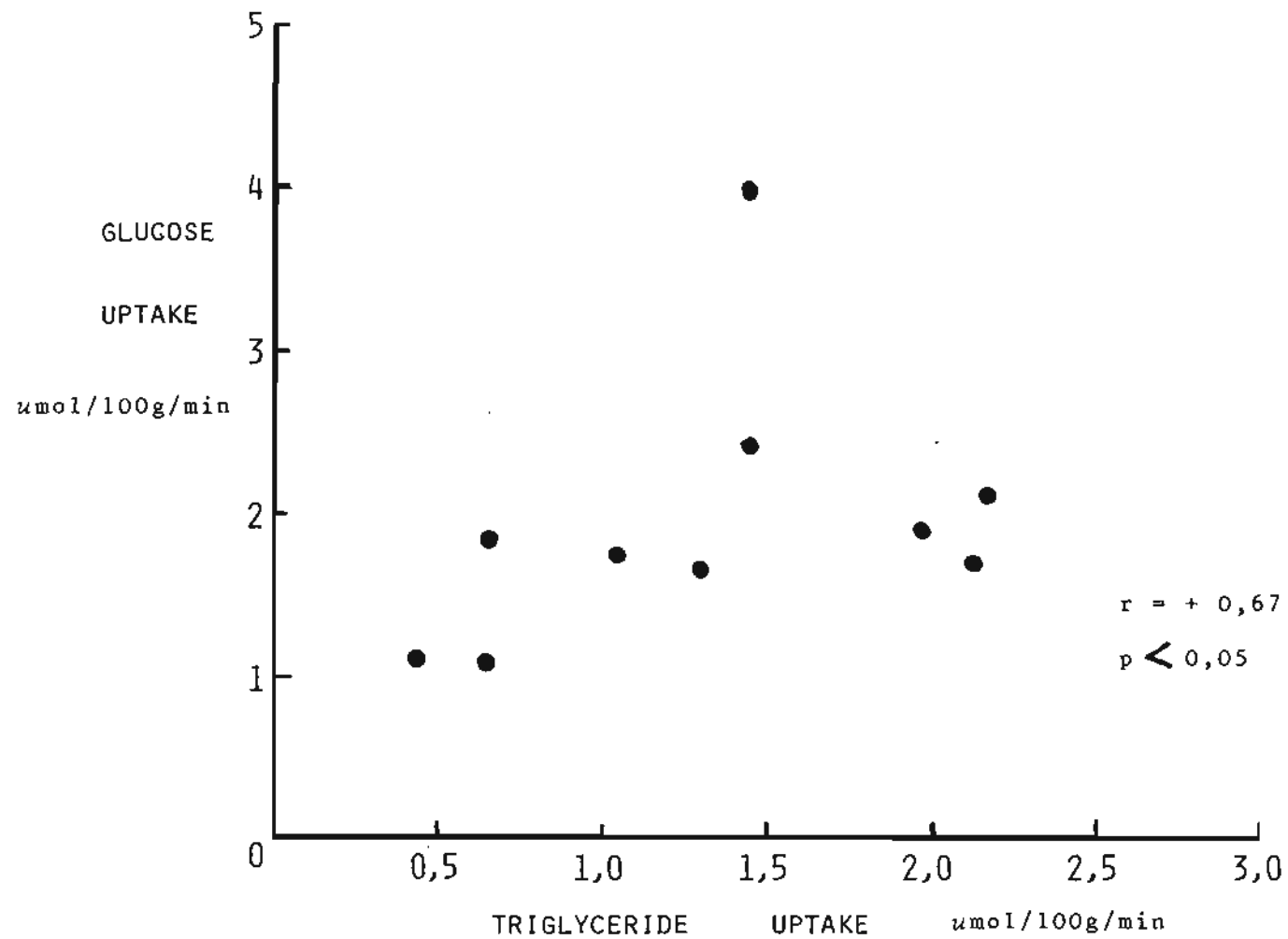


FIGURE 7.10 : CORRELATION BETWEEN GLUCOSE UPTAKE AND TRIGLYCERIDE UPTAKE IN THE NORMAL BABOON.

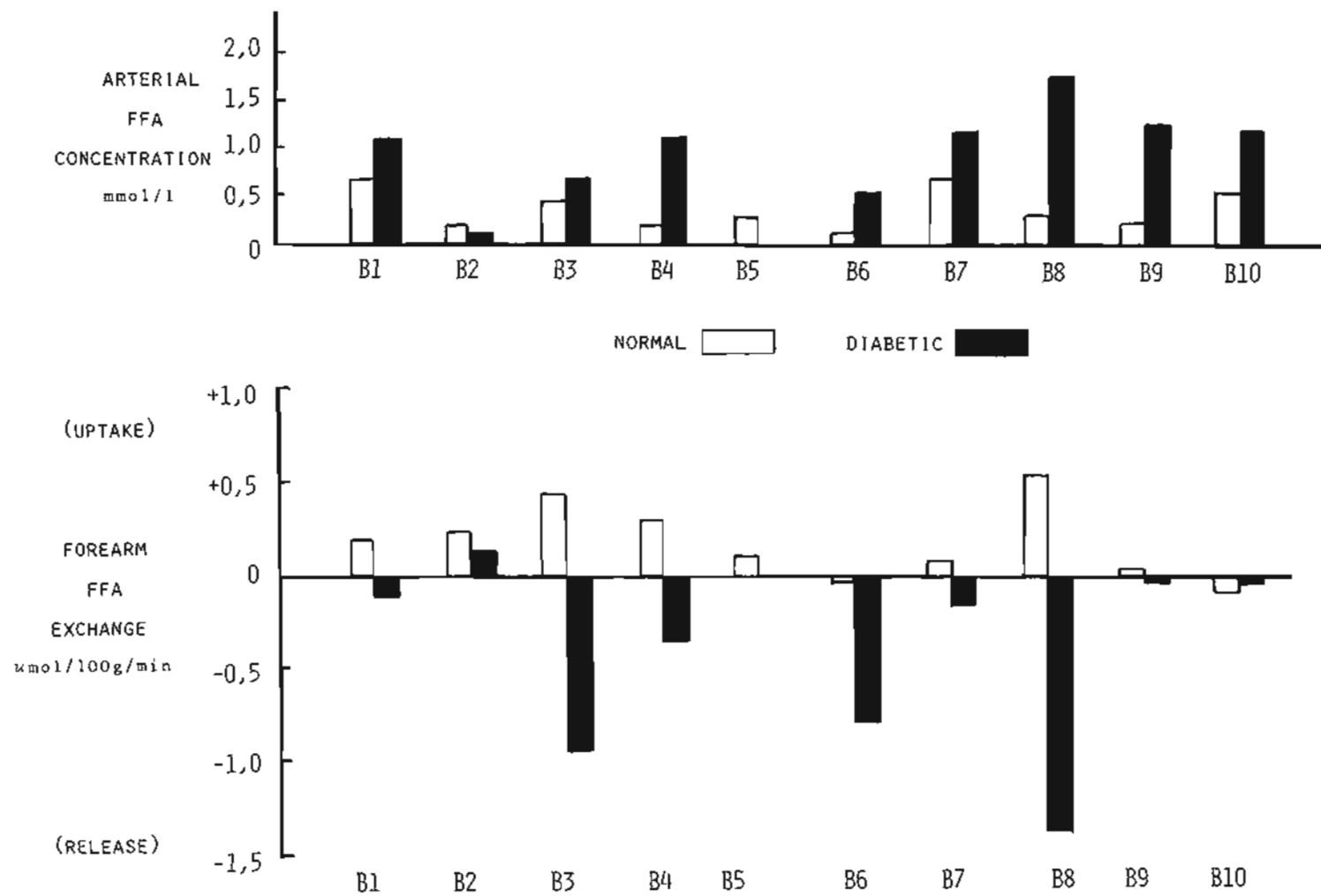


FIGURE 7.11 : ARTERIAL CONCENTRATIONS AND FOREARM EXCHANGE OF FFA BEFORE AND AFTER DIABETES.

concentration and release of FFA in the forearm of the diabetic baboon (Figure 7.12).

Glycerol: The arterial free glycerol concentration and nett forearm exchange of free glycerol in each baboon, before and after diabetes, are illustrated in Figure 7.13.

The mean arterial free glycerol concentration in the normal baboon was  $0,22 \pm 0,01$  mmol/l, and after streptozotocin it increased significantly ( $p < 0,001$ ) to  $0,46 \pm 0,04$  (Figure 7.2). The increase occurred in all baboons except B2, whose glycerol level was not significantly changed (Figure 7.13).

Significant positive correlations were found between arterial free glycerol and triglyceride concentrations (Figure 7.14) and between arterial glycerol and FFA concentrations (Figure 7.15), both before and after diabetes.

In two of the normal baboons a small uptake of glycerol was observed. In the remaining eight baboons there was a nett release of glycerol in the forearm (Figure 7.13). Overall, there was a nett release of  $0,37 \pm 0,12$   $\mu\text{mol}/100\text{g}/\text{min}$  (Figure 7.2). After streptozotocin a nett uptake of glycerol was observed in four of the baboons while the remaining five showed a nett release (Figure 7.13). Mean exchange in the diabetic baboon was an overall release of  $0,12 \pm 0,52$   $\mu\text{mol}/100\text{g}/\text{min}$  (Figure 7.2). The change in the pattern of glycerol exchange in the forearm after diabetes was not statistically significant.

## DISCUSSION

Forearm Blood Flow: Mean forearm blood flow in the normal baboon ( $5,1$  ml/100ml/min) is higher than values reported in man. With the dye dilution technique blood flow in the normal human forearm was reported



to be 3,71 ml/100ml/min (Baltzan *et al.* 1962) and 4 ml/100ml/min (Hagenfeldt and Wahren, 1972). Using a strain gauge plethysmograph to determine forearm blood flow in man, Unni and Bovill (1972) reported a flow of 4,8 ml/100ml/min, and Fauca and Hopkins (1971) using the same technique found a flow of 4,3-4,9 ml/100ml/min.

In the studies on man forearm bloodflow was determined in conscious subjects, while in the present study sedation was necessary prior to the experiments. The effects of the drugs used to produce sedation (Sernylan and Sagatal) on forearm flow are not known. Alterations in vasomotor tone resulting from the central or peripheral actions of these drugs are possible. Halothane, nitrous oxide and thiopentone have been shown to produce a significant increase in blood flow to the hand which is rapid in onset (Pauca and Hopkins, 1971). Ketalar (ketamine hydrochloride) has been shown to produce a more than twofold increase in forearm blood flow (Unni and Bovill, 1972). The possibility of an increase in the forearm blood flow after sedation of the baboon must therefore be borne in mind. Unfortunately the natural ferocity of the animal precludes estimation of forearm blood flow by plethysmography in a conscious baboon.

After the production of diabetes the forearm blood flow increased to nearly twice the normal values (Figure 7.1). Spitzer and Hori (1969) reported a similar increase in leg blood flow in the alloxan diabetic dog while Nielsen (1972) reported a 50% increase in forearm blood flow during ketoacidosis in juvenile diabetics. In contrast to these reports Munck *et al.* (1966) found no significant difference in skeletal muscle blood flow between diabetic and healthy subjects, while in the study by Ganda *et al.* (1971) mean forearm blood flow in young ketosis-prone diabetics was 50% of that in normal control subjects.

The variable results probably reflect differences in methodology as well as subject variation with regard to the duration and severity of diabetes. In human subjects with spontaneous diabetes the metabolic abnormalities have usually been present for many years and the presence of varying degrees of peripheral vascular disease, although not clinically apparent,

cannot be excluded. Experimental diabetes is produced within twenty-four hours and studies are usually made soon afterwards so that there is little possibility of the development of vascular disease.

An association between forearm blood flow and the increase in blood lactate concentration was observed in the present study. The correlation was, however, not statistically significant ( $r = 0,53$ ;  $p > 0,10$ ). It is unlikely that the increase in blood flow was due to a single factor such as the increase in lactate concentration. Forearm blood flow can increase as a result of decreased sympathetic vasomotor tone, intermediate metabolites such as lactic acid, decreased  $PO_2$ , increased  $PCO_2$ , increased hydrogen ion concentration, increased osmolarity and increased potassium ion concentration (Zelis, 1975). Many of the factors described above are often associated with insulin deficiency and it is likely that multiple factors, probably acting in a synergistic manner, are responsible for the observed increase in forearm blood flow after diabetes.

Glucose Metabolism: The mean (venous) glucose concentration in the normal baboon (*Papio ursinus*) was 4,6 mmol/l. This is similar to the mean venous glucose level of 4,5 mmol/l reported in the same species by Gillman and Gilbert (1970). The wide variation in the level of hyperglycaemia after Streptozotocin Diabetes has been discussed in Chapter Four. In insulin deprived pancreatectomised baboons fasting blood glucose ranged from 16,2 to 34,5 mmol/l (Gillman and Gilbert, 1970).

A nett uptake of glucose was observed consistently in the forearm of the normal baboon. After streptozotocin a 50% decrease in glucose uptake was observed in two baboons, while a nett release of glucose was found in the remaining seven diabetic baboons. In Table 7.1 below are shown some of the reported data on forearm metabolism of glucose in fasted normal and diabetic subjects.

TABLE 7.1 MEAN FOREARM GLUCOSE EXCHANGE (NORMAL AND DIABETIC)

Forearm Glucose Exchange ( $\mu\text{mol}/100\text{g}/\text{min}$ ) (+ = uptake; - = release)		Reference
Normal	Diabetic	
+ 1,01	+ 0,41	Asmal, 1971
+ 2,54	+ 1,41	Whichelow and Butterfield, 1971
+ 1,99	- 2,71	Ganda <i>et al.</i> 1971
+ 0,95	- 0,84	Dietze <i>et al.</i> 1975
+ 1,00*	- 0,50*	Standl <i>et al.</i> 1976
+ 1,95	- 1,65	Present study

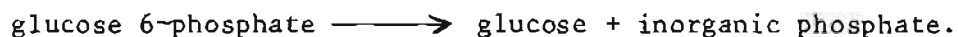
\* Approximate values taken from figure.

The observed nett uptake of glucose in the normal baboons in the present study is in keeping with observations in normal man. The fact that glucose uptake varies widely is not surprising as nett exchange is determined by several variables. These include, amongst others, the arterial glucose concentration itself, fasting insulin levels, and tissue sensitivity to insulin, and also the experimental conditions prior to and during the experiment. Forearm glucose uptake in the normal baboon correlated significantly with the arterial glucose concentration (Figure 7.4). The increased uptake with a higher arterial glucose concentration is probably chiefly the result of enhanced insulin secretion by the normal  $\beta$  cell. A correlation between glucose uptake and blood glucose concentration has been described previously (Asmal, 1971; Levine and Haft, 1970).

The present study confirms the decreased uptake of glucose reported in the forearm in diabetes in most previous studies. Glucose uptake correlated inversely with the severity of hyperglycaemia (Figure 7.5). The mild hyperglycaemia in baboons B2 and B7 was associated with a

reduced uptake of glucose, while the greater degree of hyperglycaemia in the remaining animals was associated with a nett release of glucose in the forearm. The phenomenon of glucose release has been described previously (Table 7.1). In the study by Ganda *et al.* (1971) the pattern of forearm exchange was found to be related to the type and severity of diabetes. In maturity onset diabetes (mean fasting arterial glucose 8,9 mmol/l) they found a 42% decrease in uptake of glucose in comparison to normal controls, while in young ketosis-prone diabetics (mean fasting arterial glucose 15,7 mmol/l) a nett release of glucose was observed consistently.

There is no satisfactory explanation for the observed glucose release in the forearm. Intracellularly glucose exists in the phosphorylated form and the cell membrane is impermeable to phosphorylated compounds (Newsholme and Start, 1973). Cells are able to produce free glucose if they contain the enzyme glucose 6-phosphatase which catalyses the reaction:



Muscle cells do not contain this enzyme and therefore cannot produce glucose and release it into the circulation (McGilvery, 1970).

In diabetes there is decreased utilization of glucose and increased glycogenolysis (Forman and Wiringa, 1973; Newsholme and Start, 1973; Dietze *et al.* 1975). Glycogenolysis results in the formation of glucose 6-phosphate. However a small amount of free glucose is also produced directly by the hydrolytic cleavage of the 1,6-glucose linkages (at branch points in the glycogen molecule) by the debranching enzyme (amylase 1,6-glucosidase) (Newsholme and Start, 1973). Thus it is possible that the increased rate of glycogenolysis in diabetes increases the amount of free glucose produced. Together with the fact that utilization of glucose is decreased it is possible that the resulting increase in intracellular free glucose is sufficient to account for a nett release of glucose.

Thus, while the mechanism of glucose release is unclear, and its role in contributing to the hyperglycaemia equally unresolved, in terms of glucose metabolism the significant changes were the marked elevation of blood glucose level with the production of diabetes and commensurate with the rise a marked reduction in peripheral glucose utilization. The results clearly and unequivocally show that in the acute insulin deficiency state, a severe state of peripheral underutilization of glucose rapidly develops.

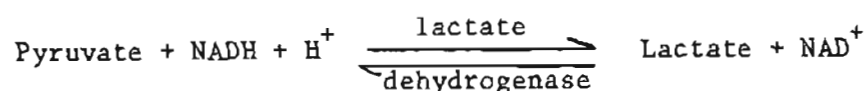
Lactate Metabolism: Mean fasting arterial lactate concentration (0,73 mmol/l) in the normal baboon is close to the reported values of 0,58 mmol/l in normal subjects (Wahren *et al.* 1975) and 0,83 mmol/l in the normal dog (Spitzer and Hori, 1969). However, it is very much lower than the average value of 2,3 mmol/l reported by de la Pena *et al.* (1973) in a different species of baboon (*Papio cynocephalus*). Although their values were probably determined in venous blood, they are nevertheless considerably higher and there is no obvious explanation for the difference.

There was a threefold increase in the mean arterial lactate concentration after streptozotocin. An increased blood lactate concentration in diabetes has been described in previous studies (Anderson and Mazza, 1963; Forbath and Hetenyi, 1970; Wahren *et al.* 1975). In contrast to these reports Spitzer and Hori (1969) reported lower arterial lactate concentrations in alloxan diabetic dogs compared to normal controls.

Nett release of lactate in the forearm was observed in all the normal baboons. The amount of lactate released correlated with glucose uptake in the normal baboon (Figure 7.7). This is not surprising as the rate of entry of glucose into cells is one of the regulatory steps in glycolysis (Newsholme and Start, 1973). Thus increased uptake will be associated with accelerated glycolysis and hence greater lactate production.

Forearm lactate release increased fourfold after Streptozotocin Diabetes. Increased production of lactate by peripheral muscle in diabetes has

been described in the rat (Berger *et al.*, 1976), the dog (Spitzer and Hori, 1969) and in man (Wahren *et al.*, 1975). The formation of lactate from pyruvate is described by the equation below:



The equilibrium constant K for the above reaction is given by the relationship

$$K = \frac{[\text{Pyruvate}] \times [\text{NADH}]}{[\text{Lactate}] \times [\text{NAD}^+]}$$

By rearrangement of the above equation

$$[\text{Lactate}] = [\text{Pyruvate}] \times \frac{[\text{NADH}]}{[\text{NAD}^+]} \times \frac{1}{K}$$

Thus increased formation of lactate can result either from increased pyruvate concentration or an increase in the cytoplasmic NADH/NAD<sup>+</sup> ratio.

Elevated blood pyruvate levels have been reported in diabetes (Anderson and Mazza, 1963; Moorhouse, 1964; Söling, 1972). The increased concentration could result from impaired oxidation of pyruvate (Hennig *et al.*, 1975; Hagg *et al.*, 1976) and probably also from increased conversion of some amino acids (e.g. serine) into pyruvate (McGilvray, 1970). An increase in the cytoplasmic NADH/NAD<sup>+</sup> ratio in liver cells in alloxan diabetes has been reported (Newsholme and Start, 1973). However, Garland and Randle (1964) have reported a decreased cytoplasmic NADH/NAD<sup>+</sup> ratio in the diaphragm and cardiac muscle cells of diabetic rats. Thus the role (if any) of the cytoplasmic NADH/NAD<sup>+</sup> ratio in diabetes in influencing lactate production by skeletal muscle is not clear.

Thus while the increase in lactate production could be ascribed mainly to the increase in pyruvate levels, the possibility of an increased cytoplasmic NADH/NAD<sup>+</sup> ratio favouring conversion of pyruvate into lactate cannot be completely excluded.

Lipid Metabolism: Fasting triglyceride concentrations vary widely in different species (Table 7.2 below).

TABLE 7.2      NORMAL FASTING TRIGLYCERIDE LEVELS IN DIFFERENT SPECIES.

Species	Triglyceride (mmol/l)	Reference
Man	0,8 - 1,8	Bold and Wilding, 1976
Rat	0,31	Corder and Kalkhoff, 1969
Sheep	0,14	Jarret <i>et al.</i> , 1972
Baboon ( <i>P. cynocephalus</i> )	0,46	de la Pena <i>et al.</i> , 1973
Baboon ( <i>P. ursinus</i> )	0,71	Present study

Primate species have lower triglyceride levels than man (Howard, 1975) and hypertriglyceridaemia can therefore only be defined relative to the norm of each particular primate species. Hence the threefold increase in mean triglyceride concentration observed in the baboon in the present study is particularly significant. Elevation of fasting triglyceride levels in diabetes is well established (Asmal, 1971; Stout *et al.* 1975).

A significant correlation was observed between arterial triglyceride and FFA (Figure 7.9) in the normal baboon. *In vitro* studies on normal and diabetic livers have shown that hepatic triglyceride synthesis and output is directly related to the concentration of FFA presented to the liver (Heimberg *et al.* 1966). Thus high arterial FFA concentrations will be associated with higher rates of hepatic triglyceride synthesis and output resulting in higher arterial triglyceride concentrations. The correlation observed *in vivo* in the present study therefore is in agreement with the observations *in vitro*.

In Table 7.3 below the fasting FFA concentration in the normal baboon is compared with values reported in other species.

TABLE 7.3      NORMAL FASTING FFA LEVELS IN DIFFERENT SPECIES.

Species	FFA ( $\mu\text{mol/l}$ )	Reference
Man	0,59	Ganda <i>et al.</i> , 1971
Rat	0,53	Corder and Kalkhoff, 1969
Sheep	0,14	Jarret <i>et al.</i> , 1972
Baboon	0,37	Present study

The arterial FFA concentration increased nearly threefold after Streptozotocin Diabetes. Increased FFA levels in diabetes is well documented and has been described in several species including the rat (Corder and Kalkhoff, 1969), dog (Basso and Havel, 1970), sheep (Jarret *et al.*, 1972) and man (Ganda *et al.*, 1971).

In Table 7.4 below the fasting glycerol level in the normal baboon is compared with values reported in other species.

TABLE 7.4      NORMAL FASTING GLYCEROL LEVELS IN DIFFERENT SPECIES.

Species	Glycerol ( $\mu\text{mol/l}$ )	Reference
Rat	210	Corder and Kalkhoff, 1969
Sheep	54	Jarret <i>et al.</i> , 1972
Man	120	Diem and Lentner, 1970
Baboon ( <i>P. cynocephalus</i> )	80	de la Pena <i>et al.</i> , 1973
Baboon ( <i>P. ureinus</i> )	220	Present study



The wide variation in normal values again emphasises the importance of intra-species and preferably intra-animal comparisons in evaluating metabolic abnormalities. After streptozotocin there was a twofold increase in the mean arterial glycerol concentration in the baboon. Increased glycerol levels in diabetes have been reported previously (Corder and Kalkhoff, 1969; Basso and Havel, 1970; Wahren *et al.*, 1975).

A significant correlation was found between arterial glycerol and triglyceride levels, both before and after diabetes (Figure 7.14). In the normal baboon serum triglyceride is hydrolysed by the enzyme lipoprotein-lipase (associated with the capillary endothelial cells) with resulting uptake of FFA and release of glycerol into the blood. Thus the association of higher levels of glycerol with higher triglyceride levels is probably the result of quantitatively greater triglyceride hydrolysis by lipoprotein-lipase. In the diabetic baboon, however, lipoprotein-lipase activity is reduced (p.110) and hence the association of high glycerol levels with high triglyceride levels cannot be accounted for on the basis of increased hydrolysis. It is possible that in the diabetic animal the reverse relationship is more likely i.e. increased glycerol levels result in its increased hepatic uptake and utilization in triglyceride synthesis with a resulting increase in triglyceride release into the circulation.

Arterial glycerol levels correlated significantly with FFA levels, before and after diabetes (Figure 7.15). Since both FFA and glycerol are produced by the same process in peripheral tissues (hydrolysis of triglyceride), and both are utilized by the liver for triglyceride synthesis, it is not surprising that a correlation exists between their concentrations in blood.

The pattern of forearm exchange in the normal baboon consisted of nett uptake of triglyceride and FFA and release of glycerol. This is consistent with the action of lipoprotein-lipase which catalyses the hydrolysis of triglyceride (in circulating lipoproteins) into FFA and glycerol. The resulting FFA are taken up by the cells (muscle and adipose tissue) while glycerol is released. In addition to the uptake of fatty acids originally present in triglyceride nett uptake of arterial FFA also occurs.

Triglyceride uptake was directly related to glucose uptake (Figure 7.10) in the normal baboon. Forearm glucose uptake is insulin dependent. Insulin enhances lipoprotein-lipase activity (Nikkilä *et al.* 1977) and hence triglyceride removal. Thus the correlation between glucose and triglyceride uptake was probably noted because both processes are influenced by insulin.

Nett uptake of FFA has been described in the normal forearm (Butterfield *et al.* 1965; Tancredi *et al.* 1976). However, nett release of FFA has also been reported (Haggendal *et al.* 1967; Ganda *et al.* 1971). These differences are likely to be due to variation in the adipose tissue content of the forearm and to the variable contribution of muscle and adipose tissue to a common venous drainage. The principal site of FFA release in the forearm is adipose tissue. Rabinowitz and Zierler (1962) reported a nett release of FFA only in "arterio-superficial venous" samples which drain chiefly adipose tissue. The peripheral venous concentration of FFA thus represents the "algebraic sum of FFA release (predominantly adipose tissue) and FFA uptake (almost exclusively muscle)" (Asmal, 1971). The baboon (*Papio ursinus*) has no visible subcutaneous fat in the forearm and it is likely that the observed nett FFA uptake represents predominantly muscle metabolism. However, fat cells are present between muscle fibres (Butterfield *et al.* 1965; Rabinowitz and Zierler, 1962) and their contribution to the nett exchange of FFA is not known.

The pattern of forearm exchange after Streptozotocin Diabetes was variable. Mean forearm exchange in the diabetic baboon was characterised by a nett release of triglyceride and decreased release of glycerol. The significance of these results is, however, questionable. Triglyceride release was not observed in all diabetic animals, while forearm glycerol exchange showed considerable individual variation and was not significantly changed after diabetes. Nevertheless the observed changes (absence of triglyceride uptake and decreased glycerol release) are consistent with the reported decrease in lipoprotein-lipase activity in diabetes (Bagdade *et al.* 1968; Bierman, 1972; Nikkilä *et al.* 1977). Furthermore nett release of triglyceride in the forearm of diabetics has been reported previously (Asmal *et al.* 1973; Leary *et al.* 1976). Thus the possibility is raised that peripheral release of triglyceride

may contribute to the hypertriglyceridaemia of diabetes. The nature of the triglyceride release process and its site of origin are unknown.

The nett release of FFA after Streptozotocin Diabetes is of interest. FFA release in the forearm has been reported in maturity onset diabetes (Asmal, 1971; Ganda *et al.* 1971) while a nett uptake of FFA was observed in young ketosis-prone diabetics (Ganda *et al.* 1971). It was anticipated that the uptake of FFA observed in the forearm of the normal baboon would increase after diabetes and that because of the absence of visible subcutaneous adipose tissue nett release of FFA would be unlikely. A possible explanation for the observed FFA release is that the greatly enhanced lipolysis and FFA release from fat cells present between muscle fibres was sufficient to mask increased uptake by muscle cells. An alternative possibility is that a nett release of FFA occurred directly from muscle cells. Dagenais *et al.* (1976), on the basis of their studies, reported that most if not all of the fatty acids oxidised by muscle are supplied by intramuscular lipid pools which are replenished by simultaneous uptake of FFA from plasma. Thus it is conceivable that the greatly enhanced hydrolysis of intramuscular triglyceride (because of insulin deficiency) makes available large amounts of FFA intracellularly, resulting in both increased oxidation and a nett efflux of FFA from the cell.

An inverse correlation was found between FFA exchange and the arterial lactate concentration (Figure 7.12) in the diabetic baboon. A similar relationship has been described in pancreatectomised dogs (Miller *et al.* 1964). There is no obvious explanation for the relationship. Increased oxidation of FFA has been reported to inhibit pyruvate utilization (Garland and Randle, 1964). Thus it is possible that high rates of oxidation of FFA are associated with a relatively smaller release of FFA and greater inhibition of pyruvate utilization. The accumulation of pyruvate would result in increased formation and release of lactate into the blood. The conversion of pyruvate to lactate would be favoured by the increased  $\text{NADH/NAD}^+$  ratio resulting from the high rate of FFA oxidation. However, the converse explanation that high blood lactate levels in some unknown way, directly or indirectly, inhibits the release of FFA is also possible.

Finally, it must be emphasised that the arterial concentration of a substrate is determined by both hepatic and peripheral metabolism, and that A-V differences are the nett result of two interdependent processes (uptake and release) occurring simultaneously and with either predominating at a particular time. Thus metabolic patterns and interrelationships are likely to be complex and it is not surprising that "statistical significance" is sometimes not present and explanations for observations often inadequate or lacking.

#### SUMMARY

1. Forearm blood flow, which in the normal baboon is slightly higher than in the human forearm, increased twofold after Streptozotocin Diabetes. Possible reasons for the increase in flow are discussed.
2. Mean arterial glucose concentration increased fourfold after streptozotocin and peripheral utilization of glucose was markedly reduced. These findings clearly demonstrate an underutilization of glucose in the acute insulin deficiency state. The significance of glucose release in diabetes remains unclear.
3. A threefold increase in mean arterial lactate concentration occurred after diabetes. Nett release of lactate occurred in the normal forearm and increased fourfold after diabetes. Lactate release correlated with glucose uptake in the normal baboon.
4. The arterial concentrations of triglyceride, glycerol and free fatty acid increased significantly after Streptozotocin Diabetes. In the normal baboon the pattern of exchange consisted of nett uptake of triglyceride and free fatty acids and release of glycerol. After diabetes there was nett release of triglyceride and free fatty acids and decreased release of glycerol. Correlations between arterial levels and forearm exchange of these substrates is discussed.

## CHAPTER EIGHT

## GLUCOSE AND LACTATE INTERRELATIONSHIPS

The acute diabetic syndrome following streptozotocin administration resulted in significantly increased rates of production and utilization (turnover rate) of both glucose and lactate. The liver utilizes lactate and produces glucose. In the extrahepatic tissues glucose is utilized and lactate produced. The increase in lactate utilization and the increase in glucose production are therefore interrelated and reflect a disturbance primarily of hepatic metabolism.

Hepatic Glucose and Lactate Metabolism.

Several factors determine the rate of lactate utilization. The concentration in blood influences hepatic uptake and thus there is increased utilization after diabetes because of the elevation in blood lactate levels. The fraction extracted may also change. Wahren *et al.* (1972) reported increased hepatic extraction of lactate in diabetic subjects with normal blood lactate levels. Thus it is likely that in the diabetic baboon there was augmented fractional extraction of lactate by the liver, quite independently of the increase in blood lactate levels. Increased fractional extraction is the result of changes in intrahepatic metabolism due to the altered hormonal status of the diabetic animal.

Lactate is an important gluconeogenic substrate. The increased incorporation of lactate into glucose and decreased oxidation of lactate to carbon dioxide has been described in diabetes (Schichiri *et al.* 1967; Steenrod *et al.* 1966). Based on recycling data, the estimated amount of glucose derived from lactate increased from 184 to 581  $\mu\text{mol/kg/hr}$  after the production of diabetes. Thus in the normal baboon 30% (368  $\mu\text{mol/kg}$ ) of the lactate turnover was converted to glucose while in the diabetic

baboon 60% (1162  $\mu\text{mol/kg}$ ) was converted to glucose. It is assumed that the basal glucose turnover rate was not significantly changed at the time the lactate turnover rate was determined. Ideally both glucose and lactate turnover rates and recycling should have been determined simultaneously, but this was not possible. Increased conversion of lactate to glucose in diabetes has been described (Forbath and Hetenyi, 1970). The metabolic pathways by which the rest of the lactate turnover is disposed is not clear. Oxidation of lactate to carbon dioxide, although decreased in diabetes, would account for part of the turnover rate, and conversion into amino acids such as alanine is possible.

Glucose produced by the liver is derived from either hydrolysis of glycogen (glycogenolysis) or synthesis from substrates such as lactate, amino acids and glycerol (gluconeogenesis). The increased production of glucose in the diabetic baboon could therefore have resulted from accelerated glycogenolysis or gluconeogenesis or both. The relative contribution of glycogenolysis to total glucose production in the baboon is not known. In normal subjects over 80% of the glucose output is derived from glycogen (Wahren *et al.* 1972) and a similar figure is likely in the baboon. Following streptozotocin administration glycogenolysis with depletion of liver glycogen occurs (Chapter Two). It is unlikely that liver glycogen content is restored to normal thereafter. The synthesis of glycogen is impaired in diabetes (Whitton and Hems, 1975) and the livers of diabetic animals are reported to have a significantly lower glycogen content (Chang, 1972; Singhal *et al.* 1971). Thus it is very likely that the fraction of the total glucose production derived from glycogen is significantly reduced in the diabetic baboon although it may still be greater than the fraction derived from gluconeogenesis.

A greatly increased rate of gluconeogenesis is the primary cause of hepatic glucose overproduction in diabetes. The amount of glucose produced from lactate increased threefold after Streptozotocin Diabetes. The activity of key enzymes involved in gluconeogenesis (pyruvate carboxylase, phosphoenol pyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase) are reported to be significantly elevated in diabetes (Forman and Wiringa, 1973; Singhal *et al.* 1971). However,

the fraction of the total glucose output derived from lactate was not significantly altered in the present study, being 20% in the normal baboon and 21% after diabetes.

About 80% of the glucose production in the diabetic baboon was derived from non lactate sources. As discussed earlier, it is likely that the contribution from glycogen was significantly reduced. Increased gluconeogenesis from other substrates could account for the remaining glucose production. The role of amino acids and glycerol in glucose production was not determined in the present study. Decreased protein synthesis and increased proteolysis are features of insulin deficiency and greatly increase the pool of amino acids available for gluconeogenesis. The metabolism of alanine, in particular its role as a gluconeogenic substrate, has become a subject of increasing interest in recent years. Augmented hepatic extraction of alanine (Wahren *et al.* 1972) and increased conversion of alanine to glucose (Chochinov *et al.* 1978) have been described in insulin dependent diabetes. Thus alanine and other glucogenic amino acids probably make a significant contribution to the increased gluconeogenesis in diabetes.

Glycerol does not appear to have a quantitatively important role in glucose production. Shaw *et al.* (1976) reported that less than 3% of hepatic glucose output was derived from glycerol in diabetic dogs although the conversion of glycerol to glucose was increased. Increased peripheral release of glycerol in the diabetic baboon probably resulted in augmented conversion into glucose by the liver. However, glucose derived from glycerol was probably an extremely small fraction of total glucose production.

The major pathway of lactate disposal in diabetes is utilization in gluconeogenesis. Thus inhibition of gluconeogenesis without a concomitant increase in lactate oxidation would result in accumulation of lactate and consequently hyperlactataemia. This is of relevance in the management of diabetes, particularly in patients on treatment with biguanides such as phenformin. The hypoglycaemic action of phenformin is due primarily to its inhibitory effect on gluconeogenesis

(Alberti and Natrass, 1977) and thus it is not surprising that hyperlactataemia is well documented in patients treated with this drug. It must be emphasized that hyperlactataemia and "lactate acidosis" are two separate entities, and while the latter is sometimes associated with the former other mechanisms are also involved in its development (Zilva, 1978).

#### Extrahepatic and Hepatic Glucose Relationships.

In the diabetic animal glucose utilization was decreased or absent in the periphery while lactate production increased. Glucose release by forearm tissues was observed in the majority of animals. The significance of these findings has been discussed in Chapter Seven. The role, if any, of the observed peripheral glucose release in the development or maintenance of hyperglycaemia in the baboon is not clear. There is little doubt, however, that abnormal peripheral (forearm) glucose metabolism is an immediate consequence of the insulin deficient state following streptozotocin administration. The abnormality in forearm glucose metabolism was noted in all diabetic baboons, whether studied at 48 or 96 hours after streptozotocin, and is of equal relevance as the disturbance in hepatic glucose metabolism to the development of the acute diabetic syndrome.

Glucose utilization in forearm tissues was reduced or absent. The increase in glucose turnover rates after diabetes is by definition an increase in the rate of production and utilization of glucose in the animal. Several factors may account for the apparent discrepancy between peripheral and whole body glucose utilization. Glucose uptake by forearm tissue (predominantly muscle in the baboon) is insulin dependent and hence reduced or absent uptake is consistent with insulin deficiency. Glucose uptake in other tissues, such as the brain, nerves, red cells and renal medulla, is not insulin dependent. Thus in the presence of hyperglycaemia glucose entry into these tissues may be increased. Evidence in support of this is the marked increase in brain glycogen reported in diabetic animals and the increased activity of the enzymes in brain forming glycogen from glucose (Forman and



Wiringa, 1973). A further important aspect is the role of the kidney in glucose homeostasis in the body. In the diabetic animal the large amount of glucose filtered at the glomerulus exceeds the tubular reabsorptive capacity. The elimination of glucose in the urine represents a significant route of disposal and would in fact be measured as "utilization" in the body.

#### Extrahepatic Lactate Metabolism.

Increased glucose uptake by tissues not dependent on insulin raises the possibility of increased lactate production by these tissues. In the fasted and resting animal the brain and red cells are the major sites of lactate production (Kreisberg, 1972). It has been suggested that the brain and red cells may possess glycolytic isoenzymes which are not insulin sensitive (Forman and Wiringa, 1973). Lactate production from glucose by the brain and red cells may therefore be increased in diabetes in addition to the observed increased production by the forearm (muscle).

The increased lactate production by extrahepatic tissues resulted in an increased lactate pool and elevated blood lactate and was associated with augmented hepatic utilization of lactate for glucose production.

#### Glucose and Lactate Pool.

Lactate pool increased by 57% and the glucose pool by 169% after the production of diabetes. The arterial lactate level increased by 215% and the arterial glucose by 318%. Thus although the increase in glucose pool was 3 times the increase in lactate pool, the elevation in blood glucose was only 1.5 times the rise in blood lactate. One of the reasons for this is the decreased rate at which the arterial glucose level rises once the renal threshold is exceeded because of urinary glucose loss. Another factor is that the lactate space decreased by 27% while the glucose space decreased by 22% after diabetes. The

relatively lower reduction in the volume of distribution of glucose would cause a smaller elevation of blood glucose relative to lactate.

In this study aspects of hepatic and peripheral metabolism were investigated concurrently in the acutely diabetic baboon. The disturbance in hepatic and peripheral metabolism developed simultaneously as an immediate consequence of the insulin deficiency following streptozotocin administration. The metabolic abnormalities in the diabetic animal can be explained entirely on the basis of insulin deficiency. However, the possibility that glucagon might play a significant role in the development of diabetes cannot be excluded. Relative or absolute hyperglucagonaemia has been identified in all forms of endogenous hyperglycaemia both spontaneous and experimental (Unger and Orci, 1975). The actions of glucagon are opposite to that of insulin and while it is debatable whether hyperglucagonaemia is necessary for the development of diabetes there is little doubt that it would accentuate the metabolic abnormalities resulting from insulin deficiency.

#### SUMMARY.

In this chapter the hepatic and extrahepatic metabolism of glucose and lactate are discussed. Interrelationships between glucose and lactate metabolism both in peripheral and hepatic tissues are considered. The biochemical basis of the abnormalities in glucose and lactate metabolism is considered and reference made to the possible role of glucagon in diabetes.

## SUMMARY

The object of the present study was to determine simultaneously aspects of hepatic and peripheral glucose metabolism in the intact baboon. Isotopic techniques were used to study glucose turnover rates, glucose recycling, glucose pool and space, and the forearm technique to study peripheral exchange of glucose. The results obtained in the normal animals acted as reference values for each animal. Thereafter diabetes mellitus was produced with streptozotocin, a drug causing destruction of the beta cells of the pancreatic islets. Experiments were then repeated in the acutely diabetic baboon and the nature and extent of the abnormalities in glucose metabolism documented. Lactate metabolism and peripheral lipid metabolism were included in the study in order to establish any interrelationships with glucose metabolism and to determine the abnormalities resulting from the production of diabetes.

In the normal animal the turnover rate of lactate was greater than glucose although the lactate pool was much smaller than the glucose pool. After producing diabetes glucose turnover rates increased threefold and correlated with the severity of hyperglycaemia. A significant increase in lactate turnover rates was noted but the increase was less than in the case of glucose turnover rates. The formation of glucose from lactate increased significantly but the fraction of the lactate turnover rate converted to glucose was unchanged. The glucose pool increased nearly threefold and correlated with the increase in glucose turnover rate. There was a significant but smaller increase in lactate pool which correlated with the increase in lactate turnover rate. Both glucose and lactate space decreased after diabetes but the decrease did not correlate with the severity of hyperglycaemia. In the majority of diabetic animals there was no glucose utilization in the forearm, and in fact glucose release was observed. Increased production of lactate occurred in the forearm of the diabetic baboon, despite decreased glucose utilization.

Arterial levels of triglyceride and free fatty acid increased threefold after diabetes while the free glycerol level doubled. In the normal animal the general pattern of exchange in the forearm consisted of triglyceride and free fatty acid uptake and free glycerol release. In the diabetic animal triglyceride and free fatty acid release was observed, while the release of free glycerol was decreased. The pattern of forearm metabolism in the diabetic animals was variable and not as consistent as before the production of diabetes. Several interrelationships between glucose, lactate and lipid metabolism were noted.

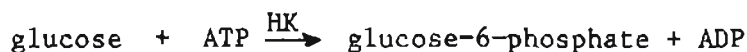
The baboons used in this study showed extreme sensitivity to the metabolic effects of Streptozotocin Diabetes. Hyperglycaemia increased in severity and ketoacidosis invariably developed in the second week. The animals were not treated with insulin and death from severe uncontrolled diabetes occurred in nearly all animals within two weeks.

This study has demonstrated the severe abnormalities in hepatic and peripheral glucose metabolism in diabetes. The simultaneous pathogenesis of these abnormalities and their importance in the development of the acute diabetic syndrome have been defined. Associated abnormalities in lactate metabolism and lipid metabolism have also been documented.

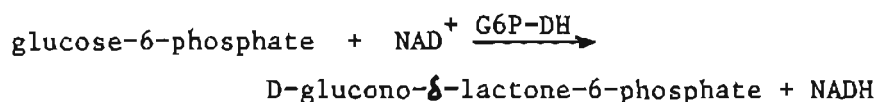
## APPENDIX ONE

BLOOD GLUCOSE DETERMINATION

Principle: The enzyme hexokinase (HK) catalyses the following reaction:



In the presence of  $\text{NAD}^+$ , the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) oxidises glucose-6-phosphate to glucono- $\delta$ -lactone-6-phosphate:



The increase in the NADH concentration is directly proportional to the glucose concentration.

Reagents:

Reagent Solution

Adenosine triphosphate	>	1 mmol/l
Nicotinamide-adenine dinucleotide	>	600 $\mu\text{mol/l}$
Hexokinase	>	140 U/l
Glucose-6-phosphate dehydrogenase	>	160 U/l
Triethanolamine buffer, pH 7,5	>	1,5 mol/l

Deproteinization Reagent

Perchloric acid		0,33 mol/l
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Procedure:

	Test	Blank	Control
Deproteinization Reagent	1,0 ml	-	1,0 ml
Specimen (Blood)	100 $\mu\text{l}$	-	-
Control Serum	-	-	100 $\mu\text{l}$
<hr/>			
Mix well. Centrifuge for 10 minutes and pipette supernatant			
Supernatant	50 $\mu\text{l}$	-	50 $\mu\text{l}$
Reagent Solution	1,0 ml	1,0 ml	1,0 ml
Deproteinization Reagent	-	50 $\mu\text{l}$	-

Mix well and after 20 minutes measure absorbance A against the blank in a spectrophotometer at 340 nm.

Calculation: Glucose (mmol/l) = A x 39,0

Column Eluate Glucose Estimation

The deproteinization step is omitted and 20 ul eluate is used directly in the same way as the supernatant.

	Test	Blank	Control
Specimen (column eluate)	20 $\mu$ l	-	-
Control solution	-	-	20 $\mu$ l
Distilled water	-	20 $\mu$ l	-
Reagent solution	1,0 ml	1,0 ml	1,0 ml

After 20 minutes the absorbance A is measured against the blank in a spectrophotometer at 340 nm.

Calculation: Glucose (mmol/l) = A x 8,1

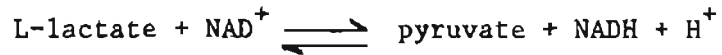
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Reference: Roche Diagnostica, 1976.

## APPENDIX TWO

BLOOD LACTATE DETERMINATION

Principle: L-lactate is oxidised to pyruvate by  $\text{NAD}^+$  in a reaction catalysed by lactate dehydrogenase (LDH).



The equilibrium of the reaction lies far to the side of L-lactate and  $\text{NAD}^+$ , but can be moved completely in the direction of pyruvate and NADH by an excess of  $\text{NAD}^+$  and the trapping of any pyruvate formed with hydrazine.

The NADH formed during the reaction is equivalent to the L-lactate present and is assayed spectrophotometrically by its absorption at 340 nm.

Reagents: Buffer/Hydrazine solution (0,5 M glycine buffer, pH = 9,0; 0,4 M hydrazine)  
Nicotinamide adenine dinucleotide ( $\text{NAD}^+$ )  
Lactic dehydrogenase (LDH) 2 mg/ml  
A working solution consisting of 50 mg  $\text{NAD}^+$ , 9,3 ml Buffer/Hydrazine solution, 0,25 ml LDH and 18,7 ml distilled water is prepared.

Procedure:	Test	Blank	Control
Working Solution	2,8 ml	2,8 ml	2,8 ml
Specimen (supernatant from deproteinized blood)	0,2 ml	-	-
Control solution	-	-	0,2 ml
7% Perchloric acid	-	0,2 ml	-

Stand for 45 minutes at room temperature. Measure absorbance A against the blank in a spectrophotometer at 340 nm.

Calculation: Blood Lactate ( $\mu\text{mol/l}$ ) = A x 7,27.

COLUMN ELUATE LACTATE DETERMINATION

A working solution consisting of 50 mg NAD<sup>+</sup>, 25 ml Buffer/Hydrazine solution and 0,25 ml LDH is prepared.

Procedure:	<u>Test</u>	<u>Blank</u>	<u>Control</u>
Working Solution	2,4 ml	2,4 ml	2,4 ml
Specimen (eluate)	0,6 ml	-	-
Formic acid (0,2 M)	-	0,6 ml	-
Control solution	-	-	0,6 ml

Stand for 45 minutes at room temperature. Measure absorbance A against the blank in a spectrophotometer at 340 nm.

Calculation: Eluate lactate ( $\mu\text{mol}/4 \text{ ml}$ ) = A x 3,22

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Reference: Boehringer Mannheim, 1974.



## APPENDIX THREE

PREPARATION OF COLUMNS

- Reagents:
1. BIORAD AG-1x8 (200-400 mesh),  
CHLORIDE FORM, ANION EXCHANGE RESIN.  
(Biorad Laboratories, California)
  2. AMMONIUM FORMATE : 600 ml 25-27% ammonium hydroxide  
is mixed with 400 ml distilled water. 400 ml 90%  
formic acid is mixed with 600 ml distilled water.  
Equal volumes of the above solutions are mixed,  
cooled, and adjusted to pH 6,5 by adding either of  
the solutions prepared above.
- Method:
1. The resin is mixed with distilled water and poured  
into glass columns (10 mm diameter) and allowed to  
settle under gravity, until the height of the resin  
bed is 5,5 cm.
  2. The column is washed with ammonium formate until  
chloride free (the presence of chloride in the  
effluent is detected with 5% silver nitrate).
  3. The column is washed with distilled water until  
ammonium free (the presence of ammonium in the  
effluent is detected with Nessler's reagent).

The prepared columns are kept moist with distilled water until the  
samples for separation are pipetted onto the columns.

## APPENDIX FOUR

ACTIVITY OF DOUBLE LABELLED SAMPLES

The activity (dpm) of samples containing  $^{14}\text{C}$  and  $^3\text{H}$  radionuclides was determined simultaneously by using two channels of a Packard liquid scintillation counter. In one channel ( $^{14}\text{C}$  channel) discriminator settings were chosen to exclude counts from the low energy  $^3\text{H}$  radionuclide. The discriminator settings of the other channel ( $^3\text{H}$  channel) compromised between  $^{14}\text{C}$  and  $^3\text{H}$  spectral separation (which ensures a minimum of emissions from the higher energy  $^{14}\text{C}$  radionuclide being counted in the  $^3\text{H}$  channel) and optimum counting efficiency of  $^3\text{H}$ . In order to determine the activity of double labelled samples three counting efficiencies must be known. These are (i) the counting efficiency of  $^{14}\text{C}$  in the  $^{14}\text{C}$  channel, (ii) the counting efficiency of  $^{14}\text{C}$  in the  $^3\text{H}$  channel, and (iii) the counting efficiency of  $^3\text{H}$  in the  $^3\text{H}$  channel. The counting efficiencies were determined by the external standard ratio method (see note below).

Two sets of standards (known dpm), each set containing samples quenched to a variable degree, were prepared. The standards in Set One contained the  $^{14}\text{C}$  radionuclide only and were counted in Brays solution using the settings described above. The efficiency of counting of  $^{14}\text{C}$  in the  $^{14}\text{C}$  and  $^3\text{H}$  channels was calculated:

$$\text{(Efficiency (\%))} = \frac{\text{cpm (obtained)}}{\text{dpm (known)}} \times 100$$

The standards in Set Two contained the  $^3\text{H}$  radionuclide only and were similarly counted and the efficiency of  $^3\text{H}$  counting in the  $^3\text{H}$  channel calculated. (The efficiency of  $^3\text{H}$  counting in the  $^{14}\text{C}$  channel was near zero.) The following plots were then made:

- (1) Counting efficiency of  $^{14}\text{C}$  in  $^{14}\text{C}$  channel against the external standard ratio (Figure A.1).
- (2) Counting efficiency of  $^{14}\text{C}$  in  $^3\text{H}$  channel ( $^{14}\text{C}$  overlap in  $^3\text{H}$  channel) against the external standard ratio (Figure A.1).

- (3) Counting efficiency of  $^3\text{H}$  in  $^3\text{H}$  channel against the external standard ratio (Figure A.2).

These curves are then used to determine the counting efficiency and thus the activity of samples quenched to an unknown extent. The curves were verified by counting samples of standard solutions containing a mixture of  $^{14}\text{C}$  and  $^3\text{H}$  radionuclides.

As an example, calculation of the activity of a double labelled sample is described below:-

Instrument Data printout :	Sample Number	X
	External Standard Ratio (ESR)	0,5320
	$^{14}\text{C}$ cpm	567
	$^3\text{H}$ cpm	699

- Calculation :
- (i)  $^{14}\text{C}$  counting efficiency of sample X (ESR 0,5320) in  $^{14}\text{C}$  channel = 57,2% (from  $^{14}\text{C}$  counting efficiency curve, Figure A.1).
  - (ii)  $^{14}\text{C}$  activity (dpm) of sample X =  $\frac{^{14}\text{C cpm}}{\text{Efficiency}} = \frac{567}{0,572} = 991$ .
  - (iii)  $^{14}\text{C}$  counting efficiency of sample X (ESR 0,5320) in  $^3\text{H}$  channel = 17,2% (from  $^{14}\text{C}$  overlap into  $^3\text{H}$  channel curve, Figure A.1).
  - (iv) Hence  $^{14}\text{C}$  counts in  $^3\text{H}$  channel ("spillover") =  $^{14}\text{C dpm} \times \text{Efficiency} = 991 \times 0,172 = 170$ .
  - (v) Corrected cpm of sample X in  $^3\text{H}$  channel =  $699 - 170 = 529$ .
  - (vi)  $^3\text{H}$  counting efficiency of sample X (ESR 0,5320) in  $^3\text{H}$  channel = 29%.
  - (vii)  $^3\text{H}$  activity (dpm) of sample X =  $\frac{^3\text{H cpm}}{\text{Efficiency}} = \frac{529}{0,29} = 1824$ .

The calculation of the activity of samples containing  $^{14}\text{C}$  only (lactate kinetics) involves steps (i) and (ii) only.

Note: External Standard Ratio

A source of gamma radiation is automatically positioned adjacent to each

vial being counted. The radiation from this external standard produces scintillations in the vial which are analysed in two specially established counting channels in the instrument. The counts ratio of the external standard in these two channels (external standard ratio) is automatically computed and printed with the rest of the sample data. The ratio correlates with the efficiency of counting of a sample quenched to an unknown degree.

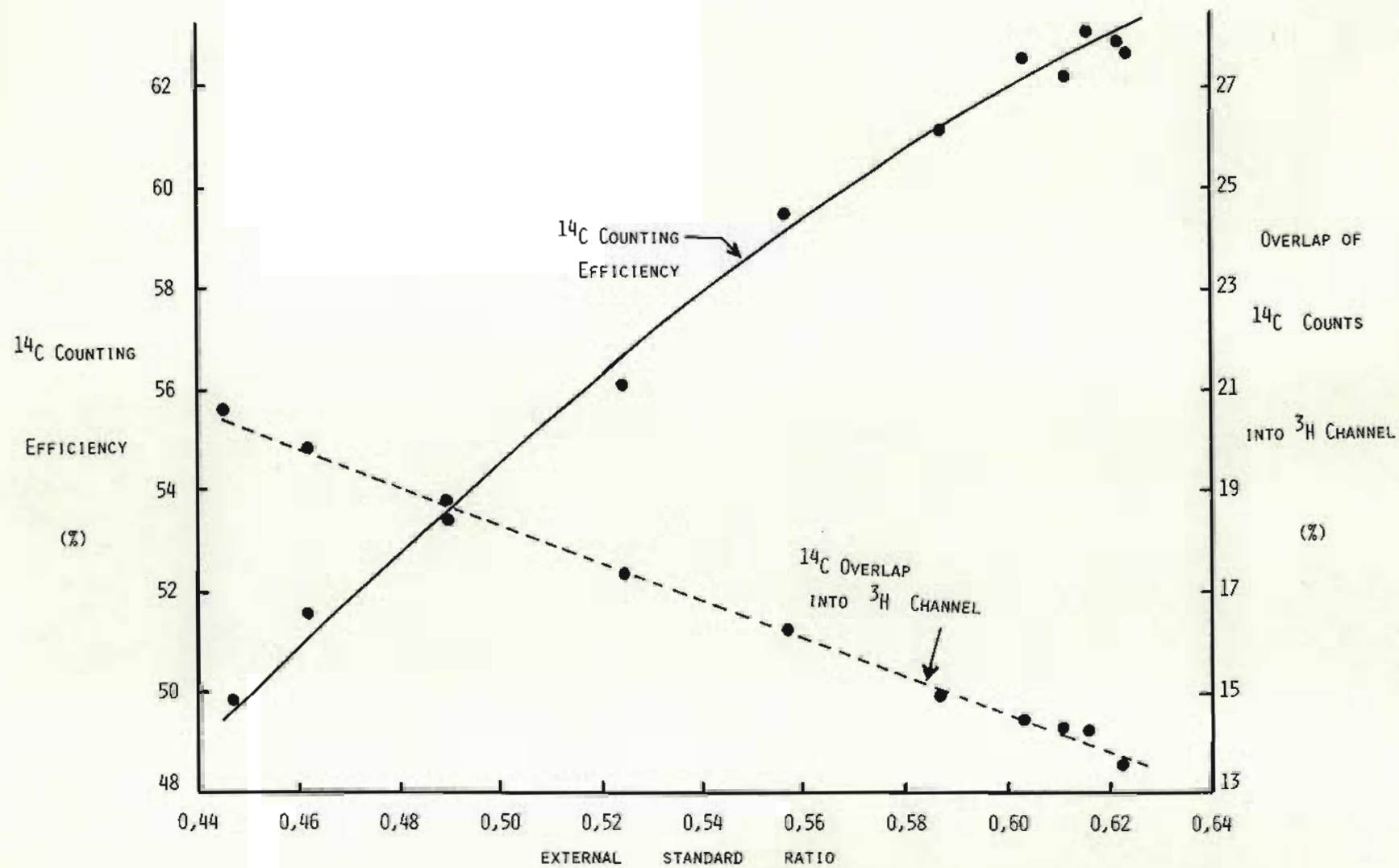


FIGURE A.1 : RELATIONSHIP BETWEEN THE EXTERNAL STANDARD RATIO AND -  
 (I) THE  $^{14}\text{C}$  COUNTING EFFICIENCY AND  
 (II) THE OVERLAP OF  $^{14}\text{C}$  COUNTS INTO THE  $^3\text{H}$  CHANNEL.

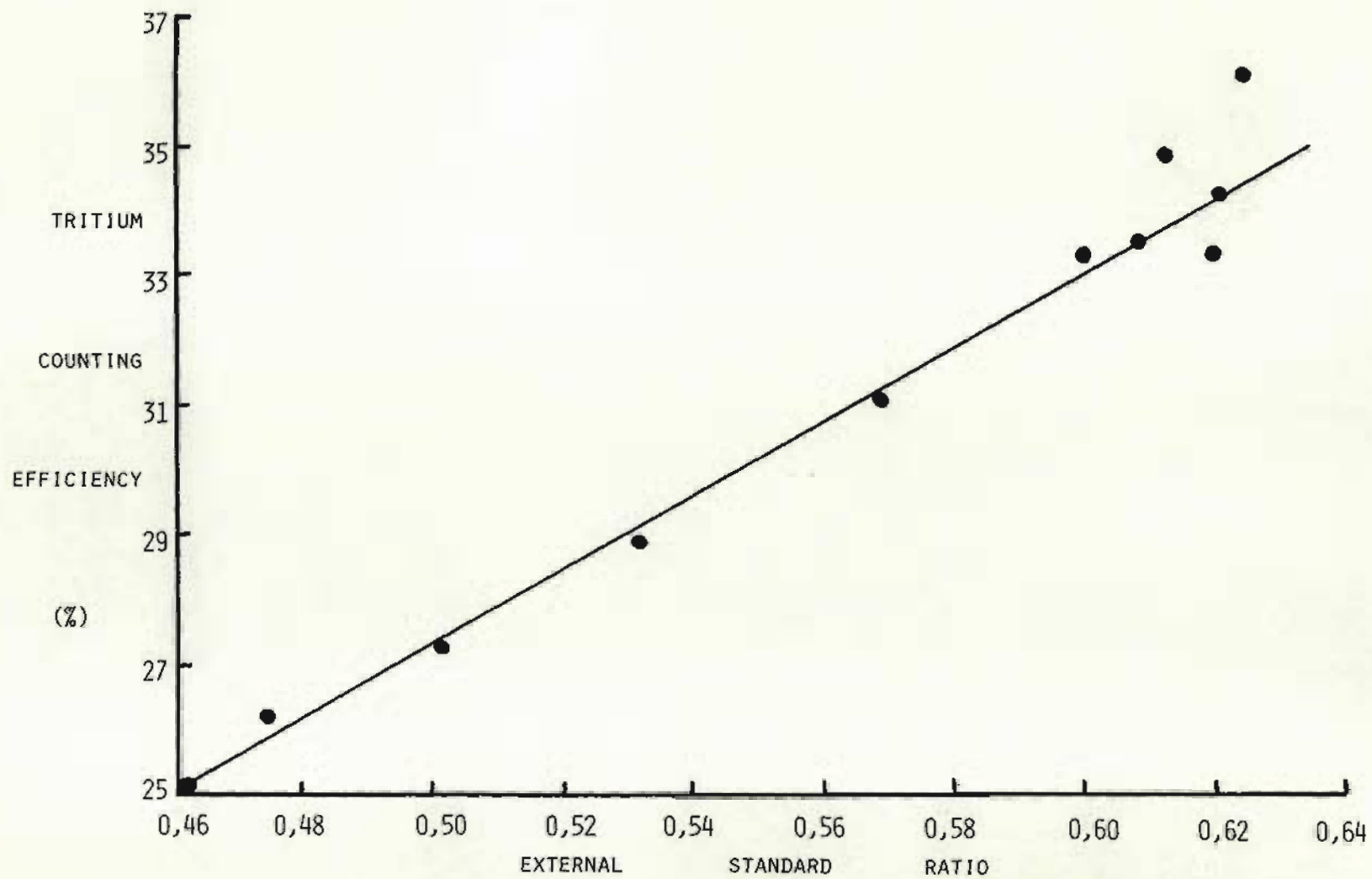
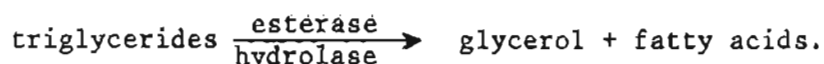


FIGURE A.2 : RELATIONSHIP BETWEEN EXTERNAL STANDARD RATIO AND TRITIUM COUNTING EFFICIENCY

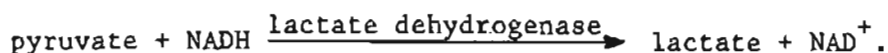
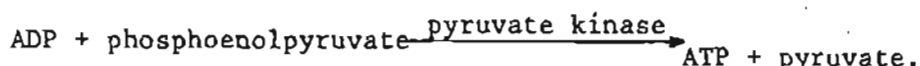
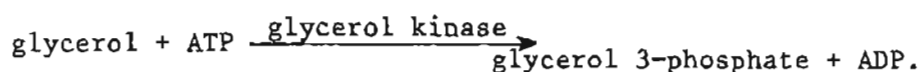
## APPENDIX FIVE

PLASMA TRIGLYCERIDE DETERMINATION

Principle: Triglycerides are split enzymatically into glycerol and free fatty acids:



The glycerol formed is subsequently determined enzymatically in a multi-stage reaction:



The decrease in the NADH concentration is directly proportional to the triglyceride concentration of the specimen and is photometrically determined.

## Reagents:

Reagent Solution (1)

Phosphate buffer/Mg <sup>++</sup> pH 7,1	44 mmol/l
Adenosine triphosphate (ATP)	175 umol/l
Phosphoenolpyruvate	250 umol/l
Esterase/hydrolase	> 12 500 U/l
Lactate dehydrogenase	> 250 U/l
Pyruvate kinase	> 150 U/l
Nicotinamide-adenine dinucleotide, reduced (NADH)	> 75 umol/l

Reagent Solution (2)

Glycerol kinase	> 55 000 U/l
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## Procedure:

	Test	Blank	Control
Reagent Solution (1)	1,0 ml	1,0 ml	1,0 ml
Specimen (Plasma)	10 $\mu$ l	-	-
Control Serum	-	-	10 $\mu$ l
Distilled Water	-	10 $\mu$ l	-

Mix well. Stand for 15 min. Measure A<sub>1</sub> against the blank in a spectrophotometer at 340 nm.

Reagent Solution (2)	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
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Mix well and incubate for 10 minutes at 30-37°C.  
Measure absorbance  $A_2$  against the blank in a  
spectrophotometer at 340 nm.

Calculation: Triglyceride (mmol/l) =  $(A_1 - A_2) \times 16,3$

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Reference: Roche Diagnostica, 1976.



## APPENDIX SIX

PLASMA FREE FATTY ACID (Non Esterified Fatty Acid ) DETERMINATION

**Principle:** The non-esterified fatty acids are converted to chloroform-soluble copper salts; the copper in the organic layer is subsequently measured colorimetrically. The concentration of non-esterified fatty acids is proportional to the absorbance of the copper-containing chloroform.

**Reagents:**Solution (1)

Triethanolamine buffer 0,45 mol/l, pH 7,8

Cupric nitrate 0,27 mol/l

Solution (2)

Diethyldithiocarbamate 9 mmol/l

Additional Reagents

Chloroform (Analytical Grade)

Fatty Acid Standard : 0,5 mmol/l

(Palmitic Acid, Analytical Grade, 12,82 mg/100 ml)

**Procedure:**

	Test	Blank	Standard	Control
Chloroform	5,0 ml	5,0 ml	5,0 ml	5,0 ml
Standard	-	-	0,2 ml	-
Control Serum	-	-	-	0,2 ml
Distilled Water	-	0,2 ml	-	-
Specimen (Plasma)	0,2 ml	-	-	-
Solution (1)	1,0 ml	1,0 ml	1,0 ml	1,0 ml

Shake vigorously for 10 minutes. Centrifuge for 5 minutes. The blue-green aqueous layer (together with the protein layer) is drawn off carefully with a fine-tipped pipette connected to a water-jet aspirator.

Chloroform layer aliquot	2,0 ml	2,0 ml	2,0 ml	2,0 ml
Solution (2)	0,2 ml	0,2 ml	0,2 ml	0,2 ml

Mix, and after 10 minutes measure absorbance A against the blank in a spectrophotometer at 420-450 nm.

Calculation: Free Fatty Acids (mmol/l) = 0,5 x  $\frac{\text{A of sample}}{\text{A of standard}}$

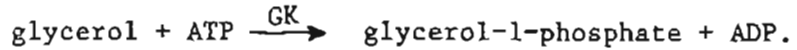
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Reference: Boehringer Mannheim, 1974.

## APPENDIX SEVEN

PLASMA FREE GLYCEROL DETERMINATION

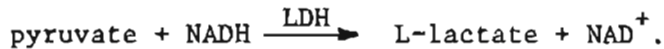
Principle: Free glycerol is converted to glycerol-1-phosphate and adenosine diphosphate (ADP) by the enzyme glycerol kinase (GK) in the presence of adenosine triphosphate (ATP):



In a subsequent enzymatic reaction with phosphoenolpyruvate (PEP), ADP is converted by pyruvate kinase (PK) to ATP and pyruvate:



In a further enzymatic reaction, the enzyme lactate dehydrogenase (LDH) catalyses the reaction of pyruvate and NADH to lactate and NAD:



The concentrations of coenzymes, enzymes and auxiliary reagents are so adjusted that the decrease in NADH concentration is directly proportional to the amount of free glycerol originally present in the sample.

Reagents:	<u>Reagent Solution (1)</u>	
	Triethanolamine buffer pH 7,6	104 mmol/l
	Adenosine triphosphate	1110 $\mu\text{mol/l}$
	Phosphoenolpyruvate	700 $\mu\text{mol/l}$
	Nicotinamide adenine dinucleotide, reduced (NADH)	495 $\mu\text{mol/l}$
	Lactate dehydrogenase	4800 U/l
	Pyruvate kinase	1050 U/l
	<u>Reagent Solution (2)</u>	
	Glycerol kinase	480 U/l

Procedure:	Test	Blank	Control
Specimen (Plasma)	0,1 ml	-	-
Reagent Solution (1)	1,5 ml	1,5 ml	1,5 ml
Control Serum	-	-	0,1 ml
Distilled Water	-	0,1 ml	-

Stand for 5 minutes. Measure absorbance  $A_1$  against the blank in a spectrophotometer at 340 nm.

Reagent Solution (2)	0,02 ml	0,02 ml	0,02 ml
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Mix well and after 10 minutes measure absorbance  $A_2$ .

Calculation: Free glycerol (mmol/l) =  $(A_1 - A_2) \times 2,6$

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Reference: Roche Diagnostica, 1976.

## REFERENCES.

- Alberti, K.G.M.M. and Nattrass, M. (1977): Lactic Acidosis. *Lancet*, 2, 25.
- Altman, P.L. and Dittmer, D.S. (Eds.) (1974): *Biology Data Book, 2nd Edition*. Bethesda: Federation of American Societies for Experimental Biology.
- Anderson, J. and Mazza, R. (1963): Pyruvate and Lactate Excretion in Patients with Diabetes Mellitus and Benign Glycosuria. *Lancet*, 2, 270.
- Anderson, T., Schein, P.S., McMenamin, M.G., Cooney, D.A. (1974): Streptozotocin Diabetes. *J. Clin. Invest.*, 54, 672.
- Andres, R., Baltzan, M.A., Cader, G., Zierler, K.L. (1962): Effect of Insulin on Carbohydrate Metabolism and on Potassium in the Forearm of Man. *J. Clin. Invest.*, 41, 108.
- Arison, R.N. and Feudale, E.L. (1967): Induction of Renal Tumour by Streptozotocin in Rats. *Nature*, 214, 1254.
- Arison, R.N., Ciaccio, E.I., Glitzer, M.S., Cassaro, J.A., Pruss, M.P. (1967): Light and Electron Microscopy of Lesions in Rats Rendered Diabetic with Streptozotocin. *Diabetes*, 16, 51.
- Asmal, A.C. (1971): *The Peripheral Metabolism of Hormones and Substrates*. M.D. Thesis, University of London.
- Asmal, A.C., Butterfield, W.H., Karamanos, B., Wichelow, M.J. (1973): Peripheral Metabolism of Triglyceride in Normal, Obese and Diabetic Subjects. *VIII Congress of the International Diabetic Federation, Excerpta Medica International Congress Series, No. 280*, 103 (Abstract 223).
- Bagdade, J.D., Porte, D., Bierman, E.L. (1968): Acute Insulin Withdrawal and the Regulation of Plasma Triglyceride Removal in Diabetic Subjects. *Diabetes*, 17, 127.

Bailey, C.C. and Bailey, O.T. (1943): The Production of Diabetes Mellitus in Rabbits with Alloxan. *J. Amer. Med. Ass.*, 122, 1165.

Baker, N., Shipley, R.A., Clark, R.E., Incefy, G.E., Skinner, S.S. (1961): <sup>14</sup>C Studies in Carbohydrate Metabolism. *Amer. J. Physiol.*, 200, 863.

Baltzan, M.A., Andres, R., Cader, G., Zierler, K.L. (1962): Heterogeneity of Forearm Metabolism with Special Reference to Free Fatty Acids. *J. Clin. Invest.*, 41, 116.

Basso, L.V. and Havel, R.J. (1970): Hepatic Metabolism of Free Fatty Acids in Normal and Diabetic Dogs. *J. Clin. Invest.*, 49, 537.

Belo, P.S., Romsos, D.R., Leveille, G.A. (1977): Influence of Diet on Lactate, Alanine and Serine Turnover and Incorporation into Glucose in the Dog. *J. Nutr.*, 107, 397.

Berger, M., Hagg, S.A., Goodman, M.N., Ruderman, N.B. (1976): Glucose Metabolism in Perfused Skeletal Muscle. *Biochem. J.*, 158, 191.

Best, C., cited in Williams, R.H. (Ed.) (1960): *Diabetes*. New York: Hoeber.

Bierman, E.L. (1972): Insulin and Hypertriglyceridaemia. *Isr. J. Med. Sci.*, 8, 303.

Boehringer Mannheim (1974): *Test Handbook*. London: Boehringer Mannheim GmbH, Biochemical Department.

Bold, A.M. and Wilding, P. (1976): *Clinical Chemistry*. London: Blackwell Scientific Publications.

Bowen, H.F. and Moorhouse, J.A. (1973): Glucose Turnover and Disposal in Maturity-Onset Diabetes. *J. Clin. Invest.*, 52, 3033.

Broder, L.E. and Carter, S.K. (1973): Pancreatic Islet Cell Carcinoma. *Ann. Intern. Med.*, 79, 108.

- Brosky, G. and Logothetopoulos, J. (1968): Streptozotocin Induced Diabetes in the Mouse and Guinea Pig. *Fed. Proc.*, 27, 547 (Abstract 1860).
- Brunschwig, A., Allen, J.G., Goldner, M.G., Gomori, G. (1943): Alloxan. *J. Amer. Med. Ass.*, 122, 966.
- Bunnag, S.C., Warner, N.E., Bunnag, S. (1967): Effect of Alloxan on the Mouse Pancreas During and After Recovery from Diabetes. *Diabetes*, 16, 83.
- Buschiazzo, H.O., Buschiazzo, P.M., Hernandez, R.E., Rodriguez, R.R. (1967): Preventive Action of Tranylcypramine on Alloxan Diabetes in Rats. *Diabetes*, 16, 810.
- Butterfield, W.J.H. and Holling, H.E. (1959): Peripheral Glucose Metabolism in Fasting Control Subjects and Diabetic Patients. *Clin. Sci.*, 18, 147.
- Butterfield, W.J.H., Hanley, H., Whichelow, M.J. (1965): Peripheral Metabolism of Glucose and Free Fatty Acids During Oral Glucose Tolerance Test. *Metabolism*, 14, 851.
- Chang, A.Y. (1972): Comparison of Liver Glycogen Synthetase Between Streptozotocin Diabetic and Control Rats. *Can. J. Biochem*, 50, 714.
- Chochinov, R.H., Bowen, H.F., Moorhouse, J.A. (1978): Circulating Alanine Disposal in Diabetes Mellitus. *Diabetes*, 27, 420.
- Christensen, N.J. and Orskov, H. (1968): The Relationship Between Endogenous Serum Insulin Concentration and Glucose Uptake in the Forearm Muscles of Non Diabetics. *J. Clin. Invest.*, 47, 1262,
- Corder, C.N. and Kalkhoff, R.K. (1969): Hepatic Lipid Metabolism in Alloxan Diabetic Rats. *J. Lab. Clin. Med.*, 73, 551.
- Dagenais, G.R., Tancredi, R.G., Zierler, K.L. (1976): Free Fatty Acid Oxidation by Forearm Muscle at Rest, and Evidence for an Intramuscular Lipid Pool in the Human Forearm. *J. Clin. Invest.*, 58, 421.
- De la Pena, A., Matthijssen, C., Goldzieher, J.W. (1973): The Baboon. *Clinical Chemistry* *Diabetes Med* 2 21

- Depocas, F., Minaire, Y., Chatonnet, J. (1969): Rates of Formation and Oxidation of Lactic Acid in Dogs at Rest and During Moderate Exercise. *Can. J. Physiol. Pharmacol.*, 47, 603.
- Diem, K. and Lentner, G. (Eds.) (1970): *Scientific Tables, 7th Edition*. Basle: Ciba-Geigy.
- Dietze, G., Wicklmayr, M., Mehnert, H. (1975): Glucose Production of Skeletal Muscle in Acute Insulin Deficiency Stimulated by Isoproterenol. *Diabetologia*, 11, 338 (Abstract 52).
- Dulin, W.E. and Wyse, B.M. (1969): Studies on the Ability of Compounds to Block the Diabetogenic Activity of Streptozotocin. *Diabetes*, 18, 459.
- Dunn, J.S. and McLetchie, N.G.B. (1943): Experimental Alloxan Diabetes in the Rat. *Lancet*, 2, 384.
- Dunn, J.S., Sheehan, H.L., McLetchie, N.G.B. (1943): Necrosis of the Islets of Langerhans Produced Experimentally. *Lancet*, 1, 484.
- Felig, P. and Wahren, J. (1971): Influence of Endogenous Insulin Secretion on Splanchnic Glucose and Amino Acid Metabolism in Man. *J. Clin. Invest.*, 50, 1702.
- Felig, P. and Wahren, J. (1975): The Liver as the Site of Insulin and Glucagon Action in Normal, Diabetic and Obese Humans. In: Shafrir, E. (Ed.), *Contemporary Topics in the Study of Diabetes and Metabolic Endocrinology*. New York/London: Academic Press Inc. p. 2.
- Forbath, N. and Hetenyi, G., Jr. (1966): Glucose Dynamics in Normal Subjects and Diabetic Patients Before and After a Glucose Load. *Diabetes*, 15, 778.
- Forbath, N., Kenshole, A.B., Hetenyi, G., Jr. (1967): Turnover of Lactic Acid in Normal and Diabetic Dogs Calculated by Two Tracer Methods. *Amer. J. Physiol.*, 212, 1179.



- Forbath, N. and Hetenyi, G., Jr. (1970): Metabolic Interrelations of Glucose and Lactate in Unanaesthetized Normal and Diabetic Dogs. *Can. J. Physiol. Pharmacol.*, 48, 115.
- Forman, D.T. and Wininga, K. (1973): Enzyme Changes in Diabetes Mellitus. *Ann. Clin. Lab. Sci.*, 3, 374.
- Freminet, A., Bursaux, E., Poyart, C.F. (1974): Effect of Elevated Lactataemia on the Rates of Lactate Turnover and Oxidation in Rats. *Pflügers Archives*, 346, 75.
- Ganda, O.P., Bhatia, M.L., Ahuja, M.M.S. (1971): Studies on Peripheral Metabolism of Glucose and Free Fatty Acids (Forearm Preparation) in Various Clinical Types of Indian Diabetics. *Indian J. Med. Res.*, 59, 1641.
- Garland, P.B. and Randle, P.J. (1964): Control of Pyruvate Dehydrogenase in the Perfused Rat Heart by the Intracellular Concentration of Acetyl Coenzyme A. *Biochem. J.*, 91, 6c.
- Gillman, J. and Gilbert, C. (1970): Some Aspects of the Endocrine Control of the Serum Lipids, Blood Ketones and Blood Sugar in Fed and Fasted Baboons, and of the Mechanism of Fuel Control in Fasting, with Special Reference to Diabetes and Lipaemia. *Exp. Med. Surg.*, 28, 45.
- Goldner, M.G. and Gomori, G. (1943): Alloxan Diabetes in the Dog. *Endocrinology*, 33, 297.
- Gomori, G. and Goldner, M.G. (1945): Acute Nature of Alloxan Damage. *Proc. Soc. Exp. Biol. Med.*, 58, 232.
- Greenfield, A.D.M., Whitney, R.J., Mobray, J.F. (1963): Methods for the Investigation of Peripheral Blood Flow. *Brit. Med. Bull.*, 19, 101.
- Gunnarsson, R., Berne, C., Hellerström, C. (1974): Cytotoxic Effects of Streptozotocin and N-nitrosomethylurea on the Pancreatic  $\beta$  Cells with Special Regard to the Role of Nicotinamide Adenine Dinucleotide. *Biochem. J.*, 140, 487.

- Hagenfeldt, L. and Wahren, J. (1972): Human Forearm Muscle Metabolism During Exercise. *Scand. J. Clin. Lab. Invest.*, 30, 429.
- Hagg, S.A., Taylor, S.I., Ruderman, N.B. (1976): Glucose Metabolism in Perfused Skeletal Muscle. *Biochem. J.*, 158, 203.
- Häggendal, E., Kerstell, J., Steen, B., Svanborg, A. (1967): Uptake of Oxygen and Substrates in Human Skeletal Muscle. *Acta Med. Scand.*, 181, 417.
- Heimberg, M., Dunkerley, A., Brown, T.O. (1966): Hepatic Lipid Metabolism in Experimental Diabetes (I). *Biochim. Biophys. Acta.*, 125, 252.
- Hennig, G., Löffler, G., Wieland, O.H. (1975): Regulation of Rat Skeletal Muscle Pyruvate Dehydrogenase Activity by Fasting, Diabetes and Work. *Diabetologia*, 11, 349 (Abstract 108).
- Herr, R.R., Eble, T.E., Bergy, M.E., Jahnke, H.K. (1959-60): Isolation and Characterization of Streptozotocin. *Antibiot. Ann.*, 7, 236.
- Herr, R.R., Jahnke, H.K., Argondelis, A.D. (1967): The Structure of Streptozotocin. *J. Amer. Chem. Soc.*, 89, 4808.
- Head, C.J., Jr. and Elrick, H. (1960): Further Studies on the Kinetics of Glucose Utilization; II, Diabetic Patients. *J. Clin. Endocrinol. Metab.*, 20, 1120.
- Hoftiezer, V. and Carpenter, A.M. (1973): Comparison of Streptozotocin and Alloxan-Induced Diabetes in the Rat, Including Volumetric Quantitation of the Pancreatic Islets. *Diabetologia*, 9, 178.
- Houssay, B.A., *et al.* (1946), cited in Rerup, C.C. (1970): Drugs Producing Diabetes Through Damage of the Insulin Secreting Cells. *Pharmacol. Rev.*, 22, 485.
- Howard, C.F., Jr. (1975): Diabetes and Lipid Metabolism in Nonhuman Primates. *Adv. Lipid Res.*, 13, 91.

- Issekutz, B., Jr., Allen, M., Borkow, I. (1972): Estimation of Glucose Turnover in the Dog with Glucose-2-T and Glucose-U-<sup>14</sup>C. *Amer. J. Physiol.*, 222, 710.
- Issekutz, B., Shaw, B.A.S., Issekutz, A.C. (1976): Lactate Metabolism in Resting and Exercising Dogs. *J. Appl. Physiol.*, 40, 312.
- Jarrett, I.G., Filsell, O.H., Ballard, F.J. (1972): Metabolic and Endocrine Interrelationships in Normal and Diabetic Sheep. In: Levine, R. and Pfeiffer, E.F. (Eds.), *Lipid Metabolism, Obesity, and Diabetes Mellitus: Impact upon Atherosclerosis. International Symposium April, 1972.* Stuttgart: Georg Thieme Publishers.
- Junod, A., Lambert, A.E., Orci, L. (1967): Studies on the Diabetogenic Action of Streptozotocin. *Proc. Soc. Exp. Biol. Med.*, 126, 201.
- Junod, A., Lambert, A.E., Stauffacher, W., Renold, A.E. (1969): Diabetogenic Action of Streptozotocin: Relationship of Dose to Metabolic Response. *J. Clin. Invest.*, 48, 2129.
- Kalant, N., Csorba, T.R., Heller, N. (1963): Effect of Insulin on Glucose Production and Utilization in Diabetes. *Metabolism*, 12, 1100.
- Kalter, S.S. (1969): Baboons. In: Beveridge, W.I.B. (Ed.), *Primates in Medicine, Vol. 2.* Basel/New York: Karger. p. 45.
- Karunanayake, E.H., Hearse, D.J., Mellows, G. (1976): Streptozotocin: Its Excretion and Metabolism in the Rat. *Diabetologia*, 12, 483.
- Katz, J. and Dunn, A. (1967): Glucose 2-<sup>3</sup>H as a Tracer for Glucose Metabolism. *Biochemistry*, 6, 1.
- Katz, J., Rostami, H., Dunn, A. (1974a): Evaluation of Glucose Turnover, Body Mass and Recycling with Reversible and Irreversible Tracers. *Biochem. J.*, 142, 161.

- Katz, J., Dunn, A., Crenoweth, M., Golden, S. (1974b): Determination of Synthesis, Recycling and Body Mass of Glucose in Rats and Rabbits *in Vivo* with  $^3\text{H}$  and  $^{14}\text{C}$ -Labelled Glucose. *Biochem. J.*, 142, 171.
- Koerker, D.J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C.J., Ensink, J., Gale, C.C. (1974): Somatostatin: Hypothalamic Inhibitor of the Endocrine Pancreas. *Science*, 184, 482.
- Kreisberg, R.A., Pennington, L.F., Boshell, B.R. (1970): Lactate Turnover and Gluconeogenesis in Normal and Obese Humans. *Diabetes*, 19, 53.
- Kreisberg, R.A., Siegal, A.M., Owen, W.C. (1971): Glucose-Lactate Interrelationships: Effect of Ethanol. *J. Clin. Invest.*, 50, 175.
- Kreisberg, R.A. (1972): Glucose-Lactate Interrelations in Man. *New Engl. J. Med.*, 287, 132.
- Lazarow, A. and Palay, S.L. (1946): Production and Course of Alloxan Diabetes in Rat. *J. Lab. Clin. Med.*, 31, 1004.
- Lazarow, A. (1947): Further Studies of Effect of Sulphur Compounds on Production of Diabetes with Alloxan. *Proc. Soc. Exp. Biol. Med.*, 66, 4.
- Lazarow, A., Liambies, J., Tausch, A.J. (1950): Protection Against Diabetes with Nicotinamide. *J. Lab. Clin. Med.*, 36, 249.
- Lazarow, A. and Patterson, J.W. (1951): Protection Against Alloxan Diabetes with Cobalt, Zinc and Ferrous Iron. *Anat. Rec.*, 109, 377 (Abstract 235).
- Leary, W.P., Asmal, A.C., Lockett, C.J., Carboni, J., Jones, S. (1976): The Effect of Sodium Pentosan Polysulphate on Peripheral Metabolism in Diabetic Patients. *IX Congress of the Int. Diabetes Fed., Excerpta Medica Int. Congress Series No. 400*, 62 (Abstract 124).

- Levine, R. and Haft, D.E. (1970): Carbohydrate Homeostasis. *New Engl. J. Med.*, 283, 237.
- Liljenquist, J.E., Mueller, G.L., Cherrington, A.D., Keller, U., Chiasson, J.L., Perry, J.M., Lacy, W.W., Rabinowitz, D. (1977): Evidence for an Important Role of Glucagon in the Regulation of Hepatic Glucose Production in Normal Man. *J. Clin. Invest.*, 59, 369.
- Little, A.D. (1966), cited in Rerup, C.C. (1970): Drugs Producing Diabetes through Damage of the Insulin Secreting Cells. *Pharmacol. Rev.*, 22, 485.
- Lundquist, I. and Rerup, C. (1967): On the Development of Alloxan Diabetes in Mice. *Eur. J. Pharmacol.*, 2, 35.
- Manougian, E., Pollycove, M., Linfoot, J.A., Lawrence, J.H. (1964): <sup>14</sup>C Glucose Kinetic Studies in Normal, Diabetic and Acromegalic Subjects. *J. Nucl. Med.*, 5, 763.
- Mansford, K.R.L. and Opie, L. (1968): Comparison of Metabolic Abnormalities in Diabetes Mellitus Induced by Streptozotocin or Alloxan. *Lancet*, 1, 670.
- McGilvery, R.W. (1970): *Biochemistry: A Functional Approach*. Philadelphia/Toronto/London: W.B. Saunders Co.
- Miller, H.I., Issekutz, B., Jr., Paul, P., Rodahl, K. (1964): Effect of Lactic Acid on Plasma Free Fatty Acids in Pancreatectomized Dogs. *Amer. J. Physiol.*, 207, 1226.
- Moorhouse, J.A. (1964): Pyruvate Tolerance Tests in Healthy and Diabetic Subjects. *Lancet*, 1, 689.
- Morgan, C.R. and Lazarow, A. (1963): Immunoassay of Insulin: Two Antibody System. *Diabetes*, 12, 115.

- Mortelmans, J. (1969): Tranquillization and Anaesthesia. In: Beveridge, W.I.B. (Ed.), *Primates in Medicine, Vol. 2*. Basel/New York: Karger. p. 113.
- Munck, O., Lindbjerg, I.F., Binder, C., Lassen, N.A., Trap-Jensen, J. (1966): Skeletal Muscle Blood Flow in Diabetic Patients Determined by Intramuscular Injection of Xenon-133. *Diabetes, 15*, 323.
- Myers, J.D. (1950): Nett Splanchnic Glucose Production in Normal Man and in Various Disease States. *J. Clin. Invest.*, 29, 1421.
- Newsholme, E.A. and Start, C. (1973): *Regulation in Metabolism*. London: John Wiley and Sons.
- Nielsen, S.L. (1972): Increased Forearm Blood Flow during Ketoacidosis in Man. *Diabetologia, 8*, 60 (Abstract).
- Nikkilä, E.A., Huttunen, J.K., Ehnholm, C. (1977): Post Heparin Plasma Lipoprotein Lipase and Hepatic Lipase in Diabetes Mellitus. *Diabetes, 26*, 11.
- Ninomiya, R., Forbath, N.F., Hetenyi, G., Jr. (1965): Effect of Adrenal Steroids on Glucose Kinetics in Normal and Diabetic Dogs. *Diabetes, 14*, 729.
- Pauca, A. and Sykes, M.K. (1967): Upper Limb Blood Flow During Thiopentone-Halothane Anaesthesia. *Brit. J. Anaesth.*, 39, 758.
- Pauca, A.L. and Hopkins, A.M. (1971): Acute Effects of Halothane, Nitrous Oxide and Thiopentone on the Upper Limb Blood Flow. *Brit. J. Anaesth.*, 43, 326.
- Paul, P. and Bortz, W.M. (1969): Turnover and Oxidation of Plasma Glucose in Lean and Obese Humans. *Metabolism, 18*, 570.
- Pitkin, R.M. and Reynolds, W.A. (1970): Diabetogenic Effects of Streptozotocin in Rhesus Monkeys. *Diabetes, 19*, 85.

Rabinowitz, D. and Zierler, K.L. (1962): Role of Free Fatty Acids in Forearm Metabolism in Man, Quantitated by Use of Insulin. *J. Clin. Invest.*, 41, 2191.

Rakieten, N., Rakieten, M.L., Nadkarni, M.V. (1963): Studies on the Diabetogenic Action of Streptozotocin. *Cancer Chemother. Rep.*, 29, 91.

Rakieten, N., Gordon, B.S., Beaty, A., Cooney, D.A., Schein, P.S. (1976a): Modification of Renal Tumorigenic Effect of Streptozotocin by Nicotinamide: Spontaneous Reversibility of Streptozotocin Diabetes. *Proc. Soc. Exp. Biol. Med.*, 151, 356.

Rakieten, N., Gordon, B.S., Beaty, A., Bates, R.W., Schein, P.S. (1976b): Streptozotocin Treatment of Streptozotocin Induced Islet Cell Adenomas in Rats. *Proc. Soc. Exp. Biol. Med.*, 151, 632.

Reichard, G.A., Jacobs, A.G., Kimbel, P., Hochella, N.J., Weinhouse, S. (1961): Blood Glucose Replacement Rates in Normal and Diabetic Humans. *J. Appl. Physiol.*, 16, 789.

Rerup, C.C. (1970): Drugs Producing Diabetes through Damage of the Insulin Secreting Cells, *Pharmacol. Rev.*, 22, 485.

Roche Diagnostica (1976): *Standardised Test Kits for the Clinical Laboratory*. Basle: F. Hoffmann - La Roche and Co., Ltd.

Schein, P.S., Cooney, D.A., Vernon, M.L. (1967): The Use of Nicotinamide to Modify the Toxicity of Streptozotocin Diabetes without Loss of Antitumour Activity. *Cancer Res.*, 27, 2324.

Schein, P.S. and Bates, R.W. (1968): Plasma Glucose Levels in Normal and Adrenalectomized Mice Treated with Streptozotocin and Nicotinamide. *Diabetes*, 17, 760.

Schein, P.S. and Loftus, S. (1968): Streptozotocin: Depression of Mouse Liver Pyridine Nucleotides. *Cancer Res.*, 28, 1501.

- Schein, P.S. (1969): 1-Methyl-1-Nitrosourea and Dialkyl Nitrosamine Depression of Nicotinamide Adenine Dinucleotide. *Cancer Res.*, 29, 1226.
- Schein, P., Kahn, R., Gorden, P., Wells, S., De Vita, V.T. (1973a): Streptozotocin for Malignant Insulinomas and Carcinoid Tumour. *Arch. Intern. Med.*, 132, 555.
- Schein, P.S., Cooney, D.A., McMenamin, M.G. (1973b): Streptozotocin Diabetes - Further Studies on the Mechanism of Depression of Nicotinamide Adenine Dinucleotide Concentrations in Mouse Pancreatic Islets and Liver. *Biochem. Pharmacol.*, 22, 2625.
- Schichiri, M., Miller, M., Woodward, J.H. (1967): Disappearance of DL-Lactate-2-C<sup>14</sup> from Blood in Normal and Diabetic Rats. *Proc. Soc. Exp. Biol. Med.*, 125, 1238.
- Searle, G.L., Strisower, E.H., Chaikoff, I.L. (1954): Glucose Pool and Glucose Space in the Normal and Diabetic Dog. *Amer. J. Physiol.*, 176, 190.
- Searle, G.L. and Cavalieri, R.R. (1972): Determination of Lactate Kinetics in the Human: Analysis of Data from Single Injection versus Continuous Infusion Methods. *Proc. Soc. Exp. Biol. Med.*, 139, 1002.
- Shames, D.M., Berman, M., Segal, S. (1971): Effects of Thyroid Disease on Glucose Oxidative Metabolism in Man: A Compartmental Model Analysis. *J. Clin. Invest.*, 50, 627.
- Shaw, W.A.S., Issekutz, T.B., Issekutz, B., Jr. (1976): Gluconeogenesis from Glycerol at Rest and During Exercise in Normal, Diabetic and Methylprednisolone Treated Dogs. *Metabolism*, 25, 329.
- Shreeve, W.W., Baker, N., Miller, M., Shipley, R.A., Incefy, G.E., Craig, J.W. (1956): <sup>14</sup>C Studies in Carbohydrate Metabolism: II Oxidation of Glucose in Diabetic Human Subjects. *Metabolism*, 5, 22.
- Singhal, R.L., Parulekar, M.R., Ling, G.M. (1971): Streptozotocin Induced Diabetes and Regulation of Hepatic Glucose Metabolism. *Can. J. Physiol. Pharmacol.*, 49, 1005.



Söling, H.D. (1972): Interrelationship Between Fatty Acid Metabolism and Hepatic Gluconeogenesis. In: Levine, R. and Pfeiffer, E.F. (Eds.), *Lipid Metabolism, Obesity, and Diabetes Mellitus: Impact upon Atherosclerosis. International Symposium April, 1972.* Stuttgart: Georg Thieme Publishers.

Spitzer, J.J. and Hori, S. (1969): Oxidation of Free Fatty Acids by Skeletal Muscle During Rest and Electrical Stimulation in Control and Diabetic Dogs. *Proc. Soc. Exp. Biol. Med.*, 131, 555.

Standl, E., Janka, H.U., Dexel, T., Kolb, H.J. (1976): Muscle Metabolism During Rest and Exercise. *Diabetes*, 25, 914.

Steele, R., Wall, J.S., De Bodo, R.C., Altszuler, N. (1956): Measurement of Size and Turnover Rate of Body Glucose Pool by Isotope Dilution Method. *Amer. J. Physiol.*, 187, 15.

Steenrod, W.J., Pahl, J.W., Barron, E.J. (1966): Metabolism of Lactate in Alloxan Diabetic Acidosis. *Diabetes*, 15, 423.

Stetten, De W., Jr., Welt, I.D., Ingle, D.J., Morley, E.H. (1951): Rates of Glucose Production and Oxidation in Normal and Diabetic Rats. *J. Biol. Chem.*, 192, 817.

Stout, R.W., Bierman, E.L., Brunzell, J.D. (1975): Atherosclerosis and Disorders of Lipid Metabolism in Diabetes. In: Valance-Owen, J. (Ed.), *Diabetes - Its Physiological and Biochemical Basis.* Lancaster: M.T.P. Press. p. 125.

Tancredi, R.G., Dagenais, G.R., Zierler, K.L. (1976): Free Fatty Acid Metabolism in the Forearm at Rest. *Johns Hopkins Med. J.*, 138, 167.

Unger, R.H. and Orci, L. (1975): The Essential Role of Glucagon in the Pathogenesis of Diabetes Mellitus. *Lancet*, 1, 14.

Unni, V.K.N. and Bovill, J.G. (1972): Forearm Blood Flow During Ketamine Anaesthesia. *Brit. J. Anaesth.*, 44, 698.

- Vavra, J.J., Deboer, C., Dietz, A., Hanka, L.J., Sokolski, W.T. (1959-60): Streptozotocin, a New Antibacterial Antibiotic. *Antibiot. Ann.*, 7, 230.
- Vondruska, J.F. (1965): Phencyclidine Anaesthesia in Baboons. *J. Amer. Vet. Med. Ass.*, 147, 1073.
- Wahren, J., Felig, P., Ahlborg, G., Jorfeldt, L. (1971): Glucose Metabolism During Leg Exercise in Man. *J. Clin. Invest.*, 50, 2715.
- Wahren, J., Felig, P., Cerasi, E., Luft, R. (1972): Splanchnic and Peripheral Glucose and Amino Acid Metabolism in Diabetes Mellitus. *J. Clin. Invest.*, 51, 1870.
- Wahren, J., Hagenfeldt, L., Felig, P. (1975): Glucose and Free Fatty Acid Utilization in Exercise. *Isr. J. Med. Sci.*, 11, 551.
- Wahren, J., Effendic, S., Luft, R., Hagenfeldt, L., Rjorkman, O., Felig, P. (1977): Influence of Somatostatin on Splanchnic Glucose Metabolism in Postabsorptive and 60-hour Fasted Humans. *J. Clin. Invest.*, 59, 299.
- Weinglass, A.R., Frame, E.G., Williams, R.H. (1945): Inhibition of Diabetogenic Action of Alloxan. *Proc. Soc. Exp. Biol. Med.*, 58, 216.
- Whichelow, M.J. and Butterfield, W.J.H. (1971): Peripheral Glucose Uptake During the Oral Glucose Tolerance Test in Normal and Obese Subjects and Borderline and Frank Diabetics. *Quart. J. Med.*, 40, 261.
- White, J.A., Bolstridge, M.C., Downing, H.J. (1974): Diabetogenic Drugs in the Vervet Monkey. *S. Afr. Med. J.*, 48, 273.
- Whitney, R.J. (1953): The Measurement of Volume Changes in Human Limbs. *J. Physiol. (London)*, 121, 1.
- Whitton, P.D. and Hems, D.A. (1975): Glycogen Synthesis in the Perfused Liver of Streptozotocin-Diabetic Rats. *Biochem. J.*, 150, 153.

Wiener, R. and Spitzer, J.J. (1974): Lactate Metabolism Following Severe Haemorrhage in the Conscious Dog. *Amer. J. Physiol.*, 227, 58.

Wohler, F. and Liebig, Von J. (1838), cited in Rerup, C.C. (1970): Drugs Producing Diabetes Through Damage of the Insulin Secreting Cells. *Pharmacol. Rev.*, 22, 485.

Wrenshall, G.A., Collins-Williams, J., Best, C.H. (1950): Initial Changes in Blood Sugar of Fasted Anaesthetized Dogs after Alloxan. *Amer. J. Physiol.*, 160, 228.

Wrenshall, G.A. and Hetenyi, G., Jr. (1959), cited in Searle, G.L. (1976): The Use of Isotope Turnover Techniques in the Study of Carbohydrate Metabolism in Man. *Clin. Endocrinol. Metab.*, 5, 783.

Zelis, R. (1975): *The Peripheral Circulations*. New York: Grune and Stratton.

Zilva, J.F. (1978): The Origin of the Acidosis in Hyperlactataemia. *Ann. Clin. Biochem.*, 15, 40.