THE ORAL GLUCOSE TOLERANCE TEST:
ROLE OF PERIPHERAL TISSUES IN THE DISPOSAL OF A STANDARD GLUCOSE LOAD

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TO MY WIFE

JUDY

"Surely there is an evil using language more precise than our knowledge. If this were the place, it would be easy to show that medicine has partaken especially of it. Its language has almost always outrun its knowledge. And the evil has been nothing less than this: our language, by ever persuading us that we are wiser than we are, has wedded us to many a capital error".

- Aphorisms from Latham, edited by William B. Bean, Iowa City, The Prairie Press.

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ABBREVIATIONS

arterialised venous	AV
arterio-deep venous	A-DV
arterio-venous	A-V
deep venous	DV
figure	Fig.
forearm glucose uptake	FGU
free fatty acids	FFA
glucose tolerance test	GTT
gram	g
growth hormone	GH
kilogram	kg
litre	l
micromole	µmol
micro unit	µU
milligram	mg
millilitre	ml
millimole	mmol
minute	min
mixed venous	MV
myocardial infarction	MI
nanogram	ng
revolutions per minute	rpm
standard error of mean	SEM
superficial venous	SV
year	yr

ABSTRACT

The studies contained in this thesis were undertaken in order to assess (1) the role of peripheral tissues in the disposal of an oral glucose load and (2) the role of peripheral glucose uptake in determining the shape of the oral glucose tolerance curve.

It is generally acknowledged that glucose uptake in skeletal muscle and adipose tissue accounts almost entirely for the peripheral disposal of an oral glucose load. Since the forearm consists predominantly of muscle and adipose tissue total forearm glucose utilisation (FGU) during the 100 g oral glucose tolerance test (GTT) was used as the index of the peripheral tissue response. FGU was determined by a simple technique involving the sampling of arterialised venous (AV) and mixed venous (MV) blood and the measurement of forearm blood flow using the mercury-in-rubber strain gauge. These investigations were carried out in normal subjects and nonobese, non-insulin requiring diabetics before and after a low carbohydrate diet. In addition, the change in concentrations across the forearm of insulin, growth hormone, lactate, acetoacetate and β-hydroxybutyrate was studied. The sampling of AV as a substitute for arterial blood was validated.

In normal subjects the increment in FGU during the three hours following glucose ingestion amounted to 74 mg/100 ml forearm; on the basis of these observations and certain assumptions it may be calculated that increased peripheral glucose utilisation accounted for the disposal of 43% of the 100 g load and that during the GTT some 58 g extra glucose reached the peripheral circulation. The

increase in FGU was fairly evenly distributed during the GTT but was maximal during the second hour. Detailed analysis of the data suggests that changes in peripheral glucose uptake have little influence on the shape of the glucose tolerance curve and that it is probably increasing hepatic glucose extraction which determines the timing of the peak glucose concentration at 30 min by preventing any further rise in glucose levels thereafter.

Following carbohydrate restriction in normal subjects glucose tolerance was significantly impaired (dietary diabetes) and the rise in serum insulin levels was frequently but not invariably, delayed. FGU, however, was not significantly decreased at any phase of the GTT or during the GTT as a whole. The results suggest that (1) the impairment of glucose tolerance following carbohydrate restriction is not the result of decreased peripheral glucose utilisation but primarily the consequence of delayed hepatic uptake of the glucose load and (2) the latter is due to a reduction in hepatic glucokinase activity rather than delayed insulin secretion.

The influence of carbohydrate restriction on the basal glucose concentration, glucose tolerance and FGU was studied in nonobese, non-insulin requiring diabetics. After carbohydrate restriction basal glucose concentrations were reduced and the glucose tolerance curve was lowered; although the increment in FGU during the GTT was increased, glucose tolerance, expressed as the incremental area under the glucose tolerance curve, remained unchanged. In addition, the responses of three diabetics on a normal diet were compared with those of age-matched normal subjects; in each diabetic FGU was equal to or greater than that in the normal subject, but despite this the increment in glucose concentrations was several

fold greater. The results suggest that in these diabetics (1) the lowering of the glucose tolerance curve by carbohydrate restriction is not synonomous with an overall improvement in tissue glucose disposal but is due primarily to a fall in the basal glucose concentration and (2) the impairment of glucose tolerance both before and after carbohydrate restriction is predominantly the result of a reduction in hepatic rather than peripheral glucose uptake. Clearly the rational interpretation of the glucose tolerance curve demands the differentiation not only of events in the periphery from those taking place in the liver but also of changes in the basal concentration from those which become apparent only after glucose loading.

Evidence is presented which indicates that the lactate, acetoacetate and β-hydroxybutyrate response curves following glucose ingestion are not determined by changes in peripheral tissues in either diabetics or nondiabetics. The findings suggest that these responses are determined at hepatic level and as such, support the conclusions reached on the basis of the glucose data.

These studies suggest that the peripheral tissue response is not the major determinant of the body's overall response to glucose ingestion i.e. while increased peripheral glucose consumption serves to minimise the increment in glucose concentrations throughout the GTT, it is primarily the hepatic response which determines the shape of the curve. The central role of the liver in determining glucose tolerance is emphasised. The interpretation of the glucose tolerance curve is discussed on the basis of these findings and recommendations made regarding the performance of the conventional glucose tolerance test.

The significant findings in this thesis are:

(1) the results suggesting that peripheral tissues do not play the major role in the disposal of an oral glucose load in normal subjects following either a normal or low carbohydrate diet

(2) the evidence showing that the rise in plasma lactate concentrations after glucose ingestion is not the consequence of increased peripheral lactate release

(3) the data indicating that the shapes of the acetoacetate and β-hydroxybutyrate response curves during the GTT are not determined at peripheral level

(4) the demonstration that AV insulin levels during the GTT significantly exceed simultaneous concentrations in MV blood and the findings of rising peripheral insulin uptake during the response.

(5) the results suggesting that a lowering of the glucose tolerance curve by carbohydrate restriction in diabetes cannot be equated with an improvement in glucose tolerance

(6) the evidence showing that at least in some men who have suffered a myocardial infarction peripheral glucose uptake is not impaired

Much of the information contained in this thesis has not been reported previously.

CHAPTER 1

AIM OF THESIS AND THE PRINCIPLES INVOLVED

The oral glucose tolerance test (GTT) remains the investigation most commonly employed in the study of carbohydrate metabolism in man. Its interpretation depends on the changes in the glucose concentrations of capillary or antecubital venous blood which follow oral glucose loading (Conn, 1958; Reaven and Miller, 1968) but since these glucose concentrations represent no more than the resultants of several metabolic processes (Steele et al., 1968), they provide no indication whatever of the processes by which they are determined. There seems little to be gained, therefore, by using the conventional GTT as the principal investigation for elucidating the mechanisms underlying a particular disturbance in glucose homeostasis, since any explanations based on the measurements of "concentrations" alone, must remain highly speculative at best. Despite this, the vast majority of workers continue to confine their studies of carbohydrate metabolism in man to the performance of conventional GTTs and as a result, the relationship between the glucose tolerance curve and the disposition of the glucose load in the body has remained poorly understood. The understanding of this relationship, however, is fundamental to the rational interpretation of the glucose tolerance curve and the work contained in this thesis is an attempt to achieve, at least in part, this understanding.

Following oral glucose challenge, the arterial glucose concentrations will depend on the rate of glucose absorption, the suppression of hepatic glucose output, the increase in hepatic glucose uptake and the augmentation of peripheral glucose utilisation in the body as a whole; the glucose concentrations in antecubital venous blood will depend, in turn, on the prevailing arterial level and the increase in glucose uptake and level of blood flow in the forearm from which blood is being sampled. It is clear, therefore that unless serial measurements are obtained of either the changes in hepatic glucose balance or peripheral glucose uptake (or both preferably)

during the GTT, the major determinants of the glucose tolerance curve will remain unknown, and the curve uninterpretable in physiological terms.

The problems associated with the determination in man of changes in hepatic glucose balance during the GTT are technically formidable and at present ethically insuperable. Firstly, cannulation of the portal vein is required and although this may be carried out by catheterisation of the umbilical vein, the procedure is neither simple nor without risk. Secondly, the administration of isotopic carbon is required; thus in order to differentiate quantitatively the two component processes which constitute hepatic glucose conservation viz. the decrease in hepatic glucose output and the increase in hepatic glucose uptake (Mebane et al., 1961; Bishop et al., 1965; Madison, 1969), C^{14}-glucose labelled in both positions 1 and 6 must be infused (Steele et al., 1968). Thirdly, since glucose ingestion induces a significant increase in hepatic blood flow (Castenfors et al., 1961; Shoemaker et al., 1963) the latter would require continual monitoring during a GTT; if, however, either Evans Blue or Indocyanine Green were to be infused in the amounts required for the relevant determinations, the subjects would acquire a distinct blue or green discolouration which would persist for several days. It is not known, furthermore, whether any other side-effects would eventuate following the infusion of dye in the quantities required. Fourthly, catheterisation of the hepatic vein would be required. Finally, it seemed hardly likely that the volunteers would maintain an emotional tranquillity throughout the necessary preparation for such a study, and since the validity of the data is dependent on the continued absence of heightened sympathetic activity (Porte, 1969) the performance of these procedures was untenable. Accordingly the direct study of hepatic glucose metabolism was not attempted.

In contradistinction to the problems associated with the study of hepatic glucose conservation, those associated with the direct study of peripheral glucose uptake are infinitely less complex and wholly acceptable within the context of human experimentation. This derives from the fact that an "aliquot" of peripheral tissues viz. the forearm, is readily available for study and furthermore, the techniques involved are simple and easily carried out (see Chapter 4). The rationale for the use of the human forearm preparation in the study of peripheral metabolism is based on its composition of muscle and adipose tissue predominantly (Abramson and Ferris, 1940; Cooper et al., 1955), on the generally accepted premise that the glucose taken up by muscle and adipose tissue accounts almost entirely for the peripheral disposal of an oral glucose load and on the assumption that the response of the forearm tissues is representative of that for like tissues throughout the body. This assumption has been accepted by a number of previous investigators of forearm metabolism (Rabinowitz and Zierler, 1962a; Butterfield and Whichelow, 1965; Felig et al., 1969), and there is little reason to doubt that, broadly speaking, it is valid. Thus it has been shown that basally, glucose uptake in the two forearms of the same individual is comparable (Whichelow et al., 1968), while in response to a given stimulus, e.g. exercise, skeletal muscle behaves similarly in terms of both glucose uptake and lactate output, whether located in the upper or lower limb (Carlson and Pernow, 1959; Donald et al., 1961; Harris et al., 1962; Sanders et al., 1964; Whichelow et al., 1968; Zierler et al., 1968).

In some instances the interpretation of conventional GTT data has been facilitated by separate studies of peripheral glucose utilisation as exemplified in the human forearm preparation. Thus, the finding by Rabinowitz and Zierler (1962a) of a reduction in the insulin-responsiveness of forearm skeletal muscle in obese subjects, has suggested that the

augmented insulin response observed during GTTs in this group (Bagdade et al., 1969) may be the consequence of increased peripheral insulin resistance. It is questionable, however, whether it is valid in all instances to use the results of insulin infusion studies, during which constant arterial glucose levels are maintained, in order to explain responses observed under the more physiological circumstances following a glucose load when the tissues are exposed to elevated concentrations of both glucose and insulin. Since the significant influence on tissue glucose uptake of increased extracellular glucose levels in their own right cannot be ignored (Lundsgaard et al., 1939; Park et al., 1959; Randle, 1963), the metabolic stimulus offered to the tissues during the GTT on the one hand and the infusion of insulin alone on the other, cannot be regarded as comparable. Indeed some of the data detailed in Chapter 6 have verified that at least in some instances equating these two circumstances may be grossly misleading.

It seemed obvious therefore that if the importance of peripheral glucose uptake in determining glucose tolerance were to be meaningfully assessed, the assessment should be carried out within the context of the conventional GTT rather than under other, less physiologically comparable, though more mathematically satisfying circumstances (Zierler, 1961). Accordingly, in this thesis I have studied forearm glucose utilisation (FGU) directly during oral GTTs with the principal aim of evaluating the role of peripheral glucose uptake in determining the characteristics of the oral glucose tolerance curve.

CHAPTER 2

THE ORAL GLUCOSE TOLERANCE CURVE: REVIEW OF CURRENT LITERATURE

(a) INTRODUCTION

All human intermediary metabolism may be subdivided, on the basis of our eating patterns, into two categories; the fed state and the fasted state. The fed state begins whenever we eat, and regulation is designed to provide efficient, rapid and synchronised disposition of exogenous fuel for oxidation and storage. When levels of circulating fuels have returned to pre-eating values, the fasted state is initiated. Simplest reproduction of the fed state with respect to carbohydrate is achieved by the performance of the GTT i.e. by the oral administration of a standard amount of glucose given as a single dose in the morning following an overnight fast and after appropriate dietary preparation during the preceding days (Conn, 1940). The resultant biphasic excursions of blood sugar may serve, therefore, as an index of the capacity for glucose disposal in the body as a whole i.e. an index of glucose tolerance. These excursions in blood sugar which constitute the glucose tolerance curve may be dissected into component parts to define where regulatory disturbances can arise. Freinkel and Metzger (1969) have published an excellent review of the subject.

(b) THE COMPONENTS OF THE GLUCOSE TOLERANCE CURVE

(1) Rising blood sugar - the "ascending limb"

Rate-limiting determinants for absorption across the intestine appear to be the delivery of the dietary mixture across the pylorus, and the active transport of glucose across the intestinal wall. The former may be prepotent. Passage of gastric contents across the pylorus is ordinarily intermittent, and only a finite proportion of the ingested load is rendered accessible to absorbing surfaces within the intestine

per unit of time. Nevertheless, although maximal transport of glucose per unit of intestinal mucosa occurs at relatively low concentrations of glucose (Wilson, 1962), the rate of delivery is such that complete absorption of glucose is effected before 100 cm of jejunum have been traversed (Bergström et al., 1957). Indeed, recent studies (Holdsworth, 1969) indicate that changes in the rate of gastric emptying or intestinal absorption are unlikely to influence significantly the oral glucose tolerance curve in intact man.

The fed state cannot be considered as initiated until the availability of insulin increases. Most of the synchronised anabolic phenomena that accompany eating are mediated by this hormone, and hence it is doubtful whether these interactions can ever be reduplicated fully in diabetic patients maintained on fixed replacement doses of insulin and incapable of acute modulations of circulating hormone. In subjects with adequate pancreatic reserve, significant increases in the insulin content of peripheral blood have been demonstrated within 10 min or less after the oral administration of glucose (McIntyre et al., 1965; Colwell and Lein, 1967; Seltzer et al., 1967). Even these may underestimate the absolute magnitude and rapidity of insulin liberation. Pancreatic insulin is released into the portal circulation, and more than half may never reach the periphery because of sequestration and perhaps ultimate degradation during its initial passage through the liver (Kaplan and Madison, 1959; Mortimore et al., 1959; Samols and Ryder, 1961). Elegant recent studies indicate that the hepatic extraction ratio for endogenous insulin is not constant and may vary with the amount of insulin that reaches the liver (Field et al., 1968). Clearly, this unresolved aspect of insulin economy must be incorporated into any meanirgful interpretation of insulin levels in peripheral blood.

Factors incident to eating and the passage of food through the gastrointestinal tract may provide the initial impetus for insulin

release. Several groups have demonstrated that oral glucose results in smaller increases in blood sugar and greater rises in plasma insulin than equivalent quantities of glucose injected intravenously (Arnould et al., 1963; Elrick et al., 1964; McIntyre et al., 1965; Perley and Kipnis, 1967). The phenomenon is not mediated by differences in hepatic "trapping" of insulin since it also occurs in subjects with porto-caval shunts in whom secreted insulin is directly delivered into the systemic circulation (McIntyre et al., 1965). A variety of gastrointestinal secretogogues such as secretin, pancreozymin and gastrin can elicit rapid and transitory increases in plasma insulin in different species when administered intravenously (Dupre and Beck, 1966; Meade et al., 1967; Unger et al., 1967; Mahler and Weisberg, 1968). The heightened vagal activity during eating may also be contributory since vagal stimulation can cause insulin release in some species such as the baboon (Daniel and Henderson, 1967). During the GTT, a major role may be exerted by glucagon that origninates from the gut wall since this hormone may stimulate insulin release (Samols et al., 1966; Unger et al., 1968). These findings corroborate the suggestion of an "entero-insular axis" advanced more than half a century ago. The relative contributions from each of the above (or other) mechanisms, however, remain to be established. Excellent historical reviews have been presented by McIntyre et al. (1965) and Unger et al. (1967).

In the presence of insulin, production of glucose from the liver is restrained almost immediately (Madison et al., 1960; Arnould et al., 1965; Steele et al., 1968). The precise mechanism of this restraint has not been elucidated. It may be linked to an acute effect of insulin on hepatic cyclic AMP (Exton et al., 1966), or potassium flux (Mortimer, 1961) since membrane barriers do not limit glucose entry into the liver cell (Cahill et al., 1958) and the activity of the insulin-dependent enzyme for phosphorylating glucose at high concentrations

of glucose (glucokinase) is not acutely enhanced by insulin (Salas et al., 1963). Net retention of an oral glucose load within the liver (that is, via restraining further glucose output and promoting intrahepatic disposition) has been estimated at about 60% in the rat (Scow and Cornfield, 1954), 20-70% in the dog (Grivas and Madison, 1964; Arnould et al., 1965; Steele et al., 1968) and as much as 70% in normal man (Perley and Kipnis, 1967). The latter estimate, however, is not based on measurements of tissue glucose uptake but only on changes in glucose concentrations during intravenous glucose administration

The glucose that is not retained within the liver gains access to the systemic circulation to increase blood sugar and to account for the ascending limb of the glucose tolerance curve. It constitutes a further stimulus to insulin secretion (Metz, 1960), and the long standing impression that liberation of insulin correlates directly with arterial glucose levels has been confirmed with perfused pancreas (Grodsky et al., 1963) and slices of pancreas (Coore and Randle, 1964). Carbohydrate metabolism of islet cells simulates many features of the liver - for example there are no membrane barriers to glucose entry (Matschinsky and Ellerman, 1968), glucose-6-phosphatase is present (Lazarus, 1959; Matschinsky and Ellerman, 1968), and a glucokinase in addition to a hexokinase has been found by some (Matschinsky and Ellerman, 1968) but not all (Ashcroft and Randle, 1968) workers. Although the release of insulin is contingent upon the phosphorylation and utilisation of glucose within the islets (Grodsky et al., 1963; Coore and Randle, 1964; Kilo et al., 1967; Ashcroft and Randle, 1968; Malaisse et al., 1968), the mechanism by which such a metabolism "triggers" the secretory response has not been clarified. It is apparent, however, that the prior and concurrent contribution of factors incident to intestinal absorption (that is, "gut factors") must make for a more immediate and sensitive response than if insulin secretion were contingent solely

upon arterial concentrations of glucose. In addition it seems likely that the response to secretory stimulation is conditioned in islets, as in other endocrine structures such as the adrenal gland, by the prevailing intracellular metabolic activity and the attendant vascularity. The in vitro finding that liberation of insulin from rabbit pancreas in response to a glucose concentration in the medium of 3 mg/ml is enhanced by supplementation of the medium with fumarate, glutamate and pyruvate (Coore and Randle, 1964) is consistent with the latter premise.

The magnitude and duration of the ascending limb of the glucose tolerance curve will obviously depend on all the above factors. A steady state is not achieved at any time. Instead, blood sugar continues to rise and to provide ever increasing stimulation to the β-cells as long as rates of intestinal absorption exceed those of glucose disposition. Throughout this period, the efficiency for the extraction and utilisation of glucose by insulin-responsive structures, such as muscle, seems to increase in parallel with the rising concentration of circulating insulin in normal subjects (Christensen and Ørskov, 1968).

(2) Peak blood sugar

When the rate of entry of alimentary glucose into the "glucose space" equals its rate of removal, the peak blood sugar is observed. It has no other physiological connotation and only represents an evanescent equilibrium during the otherwise continuous disequilibrations of the fed state.

(3) Falling blood sugar - the "descending limb"

Blood sugar falls when glucose utilisation exceeds the rate at which absorbed glucose gains access to the systemic circulation following its passage through the liver. Continuous monitoring of blood sugar

by the Auto-Analyser technique has indicated that the "descending limb" of the GTT may not be as smooth as traditionally assumed on the basis of intermittent blood sampling. Burns et al., (1965) have reported that the "peak" that occurs within the first hour in normal subjects may be followed by "second, third and even fourth maxima", of lesser magnitude, during the downward deflection of the blood sugar.

The decline in insulin parallels the glucose presumably because the direct insulinogenic stimulation of arterial glucose is diminishing. However, one additional feature is noteworthy; although glucose has returned to fasting levels by the end of two hours, the concentration of circulating immunoreactive insulin still exceeds the initial value. The significance of this phenomenon remains unexplained.

(4) The hypoglycaemic rebound

Why does blood sugar not continue to plummet downward at the end of the GTT? In vitro models with adipose tissue have indicated that insulin action ("insulinization") can persist for a finite interval after extracellular insulin is removed (Freinkel, 1964). Thus, even if much of the "extra" insulin in the circulation at this time were biologically inactive, continuing heightened utilisation of glucose by insulin-responsive tissues in the periphery might be anticipated. Moreover, autoregulation within the liver does not appear to be sensitive enough to reinstate hepatic glucose output spontaneously (Sokal and Weintraub, 1966). Clearly counter-regulatory factors must become available to antagonise glucose utilisation in the periphery and to activate hepatic glycogenolysis or gluconeogenesis or both. Whatever these factors are, the ultimate cause of the hypoglycaemic rebound would appear to be a failure of the liver to restore sufficiently rapidly an adequate level of glucose output and this is consistent with the

conclusions of Soskin and Levine (1952). Studies designed to identify the factors concerned in the re-initiation of hepatic glucose production are sparse, and an integrated reconstruction cannot be rendered at present.

Many authors have reported that plasma free fatty acids (FFA) and growth hormone increase in concert in the late phases of the GTT, sometimes even while blood sugar is still falling (Berson and Yalow,, 1965). The temporal concurrence suggests that the growth hormone cannot be responsible for the activation of lipolysis (Berson and Yalow, 1965). The late rise in plasma FFA that can be observed during the GTT in patients with isolated deficiency of growth hormone (Wilber and Odell, 1965) supports this inference. It seems more likely that the simultaneous changes in growth hormone and FFA are secondary expressions of some higher response to the "stress" of diminishing blood sugar.

An attractive although still conjunctural case can be made for a "triggering" of counter-regulation via a centre (or inter-related centres) within the central nervous system. Recently, adrenergic receptor mechanisms have been demonstrated for growth hormone (Blackard and Heidingsfelder, 1968) and insulin (Porte, 1967); effects upon α-receptors could augment secretion of growth hormone (Blackard and Beidingsfelder, 1968) and inhibit release of insulin (Porte, 1967). Goodner et al., (1967) have implicated an autonomic centre within the central nervous system in the tonic modulation of FFA and Bolinger et al. (1962) have demonstrated positive correlations between the peak values for urinary vanillylmandelic acid and the secondary rise in plasma FFA during intravenous GTTs.

Some counter-regulatory activity of hepatic glycogenolysis and gluconeogenesis and inhibition of peripheral glucose utilisation could also be mediated via pancreatic glucagon. Recent work, which obviates many of the analytical uncertainties that have plagued glucagon research,

has indicated that the pancreatic elaboration of glucagon in vivo (Ohneda et al., 1968) and in vitro (Vance et al., 1968) seems to vary inversely with the prevailing extracellular concentration of glucose.

Thus the available evidence, although incomplete, suggests that the "descending limb" of the GTT may be viewed as a miniature insulin tolerance test. On a qualitative, although not a quantitative basis, the same counter-regulatory forces may be called into play during the descent from hyperglycaemia to normoglycaemia, as from normoglycaemia to hypoglycaemia. That this counter-regulation is not perfect is shown by the fact that the blood sugar frequently exhibits a transient dip below fasting levels during the late phases of the GTT (that is, the "hypoglycaemic rebound"). When this has been documented precisely, by continuous monitoring of blood sugar, nine out of eleven normal subjects displayed nadirs ranging from 10-56 mg/100 ml below initial values (Burns et al., 1965).

CHAPTER 3

HISTORICAL REVIEW

For centuries writers have expressed their fascination with the unusual taste of the urine of diabetic subjects. Indeed, the term "honey-urine" was used in the works of early Indian authors, and ancient Chinese and Japanese writers stressed the excessive quantity and sweetness of the urine which attracted dogs and ants (Wolff, 1958). The first European author to record the sweet taste of diabetic urine was Thomas Willis (1679) who wrote "But why that it is wonderfully sweet like sugar or honey, this difficulty is worthy of explanation". A century later Matthew Dobson (1776) published his classic paper "Experiments and Observations on the Urine in Diabetes" in which he confirmed the sweet taste of the urine and demonstrated, in one patient, the presence of a substance which "readily passed through vinous, acetous and putrefactive fermentations". He also noted that the serum of this patient "was sweetish, but I thought not so sweet as the urine". Dobson (1776) evaporated the urine to dryness and obtained a white cake

> "which granulated and broke easily between the finger; it smelled sweet like brown sugar, neither could it from the taste be distinguished from sugar, except that the sweetness left a slight sense of coolness on the palate".

He concluded

> "that a considerable quantity of saccharine matter passed off by the kidneys, in this case of diabetes, and probably does so in every instance of this disease, where the urine has a sweet taste... it further appears, that this saccharine matter was not formed in the secretory organ, but previously existed in the serum of the blood".

Glucose was not recognised as a separate substance, however, until 1792 when Lowitz isolated a sugar from honey which he considered to be different from cane sugar. Eleven years later Proust (1803) confirmed this finding and demonstrated that the crystallizable sugar present in grape juice was identical with that which Lowitz had obtained from honey. In 1811 Kirchoff hydrolysed starch with dilute sulphuric acid and produced a sweet syrup from which a crystalline substance was isolated; the latter was identical with the sugar obtained from honey and grapes

and was called grape sugar. Four years later in 1815 Chevreul obtained sugar from diabetic urine in the form of little crystals, similar to those produced from grape sugar; he submitted them to a variety of procedures including recrystallization and estimated their melting point in water and alcohol but could detect no differences from grape sugar.

Despite the observations of Dobson few studies were conducted on the presence of sugar in the blood of diabetics. In 1811 Henry wrote that it had been satisfactorily proved that saccharine matter did not exist ready formed in the serum of diabetic blood and quoted his own experimental studies and those of Nicolas and Gueudeville and Wollaston. The latter, according to Bernard (1877), subsequently recognised that blood serum did contain sugar, but only one thirtieth of the amount found in an equal volume of urine; the extra sugar in the urine was thought to be the result of work performed by the kidneys. In 1836 however, Ambrosiani found sugar in the blood of a diabetic but whereas earlier workers had performed their estimations on blood which had been standing for some time, Ambrosiani (1836) worked on blood immediately after it had been taken. He used a method which involved the addition of the white of an egg and obtained a syrup which underwent fermentation and would deposit crystals of sugar. In contrast, in another diabetic Ambrosiani found no trace of sugar. In 1837 McGregor bled a diabetic patient and noted a milky serum, quite distinct from that of healthy blood, from which he obtained a fermentable solution. He confirmed this finding on other diabetic subjects and also found sugar in their saliva and stool. He wrote that he had been able to find a trace of sugar even in the blood of healthy individuals when fed upon a vegetable diet.

In 1839 Bouchardat attempted to explain the contradictory results of earlier workers regarding the presence of sugar in blood. He took blood at 9 a.m. from a diabetic who had not eaten since the previous evening and found no trace of sugar in the blood. On another occasion

he obtained unequivocal evidence of the presence of sugar in blood taken from a patient two hours after a light meal and concluded that the sugar appeared im the blood, and subsequently in the urine, as a result of the ingestion of sugar-containing foods.

Despite the earlier writings of McGregor (1837), Magendie (1846) is usually given credit as the first person to recognise that sugar could exist in the blood under physiological conditions. Previously the finding of sugar in the blood was considered a morbid phenomenon. Magendie (1846) studied the ability of various tissues and body fluids to digest starch. Following intravenous injection of starch, sugar appeared in the blood of a rabbit. He then fed a dog on a diet of potatoes and lard and found a notable amount of sugar in the blood; the urine of the animal contained no sugar. Magendie concluded that the appearance of sugar in the blood was related to the ingestion of starchy foods. In another experiment he took a pigeon which had its pancreatic duct destroyed (by Bernard) and fed it on vetch for six weeks and then killed it to obtain its blood; this contained an appreciable amount of sugar, Magendie queried how a bird deprived of salivary glands and pancreas could digest the starch. Unfortunately he concluded that it was broken down by the bile with the subsequent absorption of glucose into the circulation.

Modern physiological concepts of glucose metabolism have their origin in the studies of Claude Bernard. In his classic paper "De l'origine du sucre dans l'economie animale" presented to the Societe Biologique on the 21st October, 1848, he demonstrated the existence of sugar in the blood of animals in the absence of prior carbohydrate feeding (Bernard, 1848). In two healthy dogs sugar was present in blood taken from the heart after death. One of the dogs had been fed on a meal which contained no starch; the other animal had received no food for two days. In subsequent experiments to ascertain the origin of the

sugar Bernard placed ligatures at various places in the portal venous system as soon as possible after death. He found no glucose in the blood coming from the spleen or pancreas; sugar was present in blood draining from the intestine, but the greatest quantity of sugar was found in the blood in the intrahepatic portions of the portal vein. Bernard (1877) concluded that the sugar originated in the liver and although he initially believed that the sugar released by the liver differed from grape sugar, he subsequently acknowledged their identity. The word "glucose" was first introduced in 1838 by Dumas while in 1866 Kekule coined the term "dextrose".

For many years Bernard insisted that the portal blood of a meat-fed dog did not contain sugar. Several investigators disagreed strongly with him on this point. For example, Figuier (1855) found sugar in the portal vein of a dog two and a half hours after ingestion of meat but not in the portal blood of an animal which had been starved of food. Chaveau (1856), on the other hand, found sugar in portal blood and in the blood draining the small intestine in animals fasted for forty-eight hours. Pavy (1860) also detected sugar in portal venous blood - and in amounts similar to those found under normal circumstances in the heart and other vessels of the body.

One of the most important results of the finding of glucose in the blood of fasting animals was the discovery of glycogen, which, by Bernard's own admission, was fortuitous. Normally Bernard made duplicate estimations of the sugar content of liver tissue simultaneously but on one occasion, pressed for time, he made one estimation immediately after the death of the animal and the other was delayed until the next day. The second estimation gave a much higher result than the first and on investigating this discrepancy, he found that the time factor was of great importance in determining the amount of glucose present in the specimen i.e. that only a small amount of glucose might be present soon

after death but a large amount after about two hours. Bernard (1855a) found, furthermore, that large amounts of sugar continued to be released by a still warm liver after the tissue had been freed of sugar by perfusion with cold water, proving that the sugar was formed from material in the liver itself. Bernard (1857) went on to isolate the sugar-forming substance, which he named 'glycogen', and described many of its chemical and physical properties. In the same year Sanson (1857) found that muscle tissue also contained a considerable amount of glycogen.

Bernard's concepts of the importance of glycogen were severely criticised by Pavy (1860) since he considered Bernard's findings to be associated with the death of the animal. This criticism was based on the findings that respiratory obstruction (Bernard, 1855b, 1860) and struggling (Bernard, 1860) caused an unnatural increase of sugar in the circulation and that the sugar content of blood taken from the heart during life was considerably less (i.e. only a trace) than that found shortly after death.

The study of the fate of ingested glucose was a principal aim of Claude Bernard's investigations. In 1853 he wrote

> "My aim was to follow closely the sugar which was absorbed from the food in its passage along the blood stream, first to the liver, then to the lungs, and finally to all the other tissues in the body. I wanted to know if the sugar was destroyed in traversing the liver, which is the first organ through which it passes after being absorbed into the tributaries of the portal vein".

As pointed out by Young (1937) some of Bernard's early theories on the regulation of glucose metabolism were erroneous. He believed, for example, that the sugar liberated by the liver diffused over the organism by way of the circulation and that the amount steadily diminished with the distance of the blood from the liver (Bernard, 1855a). He also believed that nitrogenous matter was the chief source of the glycogen synthesised by the liver (Bernard, 1877) and that although

cane sugar and probably other carbohydrates were "nutritive stimulators" of glycogen formation, they themselves were not incorporated into glycogen.

Bernard's view of the role of the liver in glucose homeostasis remained confused since he seemed unable to reconcile in his own mind the existence of both glucose uptake and production as major functions in the same organ. In 1877 he wrote "In the liver sugar is produced although a little is also destroyed in that organ; in the muscles sugar is destroyed. Destruction of sugar probably occurs throughout the organism, in all the organs, in all the tissues". In the same book, however, he also wrote "The liver acts upon and transforms any sugar which appears, directly or indirectly, as a result of the absorption of food. The sugar disappears in its passage across the gland, while glycogen appears", suggesting that Bernard believed that the liver blocked the passage of sugar through it. This latter view was supported by his observations that if a given quantity of glucose was injected into the jugular vein of a dog it passed more easily into the urine than if the same quantity of glucose was injected into a tributary of the portal vein. He also found that feeding potatoes to a dog with portal vein obstruction resulted in definite glycosuria; he quoted as clinical confirmation the presence of diabetes in a subject with portal vein obstruction and alimentary glycosuria in cirrhotics with portal vein obliteration.

Pavy had quite a different concept of the fate of ingested glucose and the role of the liver (Young, 1937). He found in 1858 that the total amount of glycogen in the liver of sugar-fed aminals was much greater than that to be found in the liver of animals receiving other types of food; he therefore considered that liver glycogen night be formed from the sugar in food. In 1862 he found that the blood flowing from the liver of an animals which was digesting carbohydrate had a smaller

concentration of sugar than was to be found, on the average, in the blood of the portal vein under similar circumstances suggesting that the liver was indeed absorbing the ingested sugar (Young, 1937). Pavy (1894) also believed that the circulating blood contained a standard amount of glucose which presented no essential variation in the different parts of the system. This normal stability of the blood sugar level and the serious nature of the disease diabetes, characterised by high blood sugar levels, led Pavy to suggest in 1894 that glucose was toxic to be body and that the liver protected the organism by converting the sugar to glycogen.

The dominating factors governing research on carbohydrate metabolism were the prevalence and serious complications of diabetes mellitus. Apart from its clinical characteristics, the main diagnostic feature of diabetes remained glycosuria; it is not surprising, therefore, that in the absence of a reliable method for measuring glucose in small amounts of blood, considerable interest evolved in the quantitative relationship between glycosuria and the diabetic state. The concept of "carbohydrate tolerance" emerged at this time to denominate in general the ability of an individual to handle carbohydrate. According to Folin and Berglund (1922), this concept originally referred to the amount of carbohydrate which a diabetic patient could take in the course of twenty-four hours without the appearance of sugar in the urine. As pointed out by these authors (Folin and Berglund, 1922), the original concept of alimentary glycosuria induced by other routes of sugar administration implied the false assumption that the taking of a larger dose of sugar than that just sufficient to produce some glycosuria meant that all the surplus sugar taken would pass into the urine. The erroneous character of this assumption was clearly pointed out, in 1895, by Linossier and Rogue, who expressed the fact that "the more sugar that is taken the more is retained and utilised by non-

diabetic individuals". The inadequacy of this concept was further demonstrated in 1911 by Pavy and Godden who showed that when intravenous glucose was given the production of glycosuria depended more on the rapidity of the administration than on the dose given.

A different meaning for glucose tolerance was used after Hofmeister (1889) introduced the term "limit of sugar assimilation" to indicate the amount of sugar which could be taken in one dose without any demonstrable loss of sugar in the urine. It was to this form of measurement that Allen (1913) referred when he wrote

> "Tests of sugar tolerance may be oral, subcutaneous, or intravenous. Each may possess some advantages. The oral method holds its clinical position by reason of its convenience; it is open to error through irregularities of absorption and the role of the liver is important, especially in the case of lævulose. The intravenous test is perhaps the least useful; it is merely a kind of saturation limit, representing the amount of sugar the tissues and fluids can hold without overflowing the kidneys; undue importance may thus attach to slight variations of renal permeability; especially the test does not determine the ability of the tissues to withdraw sugar from the blood. The subcutaneous method is the best test of the power of the body-tissues to utilize sugar".

The measurement of glucose tolerance by assessment of glycosuria was always of doubtful significance due to the variables involved. In particular there was considerable argument as to whether or not glucose was present in normal urine while those who claimed that glucose was present were unable to agree on the amount which should be considered as normal. In addition, it was suggested that the blood sugar level was not the only factor determining glycosuria. Thus in 1913 Allen wrote

> "The relative permeability of the kidneys for sugar is a factor, sometimes an important factor, in determining the presence or absence of glycosuria under a wide variety of conditions. The renal factor, like other factors governing glycosuria, may produce effects in either direction, either towards causing glycosuria or towards preventing it".

The relationship between the height of the blood sugar and the presence of glycosuria had been recognised by Bernard and Pavy but later workers established the relationship more clearly. The need for

a reasonably accurate micro-method of measuring blood sugar was met by Bang in 1913. In his important monograph (Bang, 1913), which, unfortunately, was never translated into English, Bang presented a method for the determination of sugar in 0.2 ml blood or less. Almost simultaneously Lewis and Benedict (1913) described a method suitable for the analysis of about 2.0 ml blood. These two methods for the first time made blood sugar determinations practical clinical procedures. Bang's procedure was generally adopted in Europe, and became the standard on which other techniques were modelled. It was suitable for the analysis of such small amounts of blood as could be obtained by puncture of the skin of the finger or the ear lobe. Benedict's method was as generally accepted in America and subsequent methods in the United States followed Benedict's lead in requiring 1.0-2.0 ml blood, rendering them applicable only to the study of venous blood. Because of the requirements of the particular methods in vogue at this time, European investigators in general examined capillary blood while American workers sampled venous blood in their clinical studies.

With the increased sensitivity and accuracy of these methods the assessment of glucose tolerance gradually came to be determined not by the presence or absence of glycosuria but by the change in the blood sugar levels following the oral administration of glucose. Glucose tolerance thus came to indicate the degree and duration of the hyperglycaemia following a given dose of glucose - a high tolerance being the condition where the hyperglycaemia after glucose administration is small or transitory, a low tolerance where the hyperglycaemia is high or prolonged.

Three accepted types of GTT have been developed over the years; they differ mainly in the method of glucose administration i.e. intravenously or orally and, in the case of the oral test, the number

of doses employed, The intravenous test is carried out by the injection of 25 g glucose and has been used widely in some quarters. Whatever the advantages of this test, however, the intravenous route is not the normal route of entry of carbohydrate into the body and this investigation cannot be regarded as a test of the normal mecahnisms which regulate glucose homeostasis. Thus, as pointed out by Mosenthal and Barry (1950), "the rate of glucose absorption from the intestinal tract has a distinct clinical significance" while the physiological importance of the liver in the portal circulation (Madison, 1969) cannot be ignored.

The second type of test viz. the Exton-Rose two-dose, one hour test, calls for the oral administration of 50 g glucose in the fasting state and again after 30 min, blood samples being taken at 0, 30 and 60 min after the first dose of glucose. This method, proposed by Exton and Rose (1934) as a separate physiological entity, takes advantage of the Hamman-Hirschmann (Hamman and Hirschmann, 1917) or Staub-Traugott (Staub, 1921; Traugott, 1922) phenomenon viz. that after successive doses of glucose given by mouth, each successive rise in the blood sugar levels is less than the preceding one. This test went through a period of enthusiastic endorsement, then modification of standards, and finally scepticism of its validity climaxed by the reasoning of Langner et al. (1946). The latter suggested that the absorption of glucose from the gut and the response of the glucose regulating mechanism is not sufficiently rapid to permit a paradoxical response in the short space of half an hour - and this was contrary to the basis on which this test was proposed. Thus Langner et al. (1946) concluded that after giving 100 g glucose in one initial dose, the blood glucose curve was essentially the same as that obtained after giving 100 g in two divided doses of 50 g as in the Exton-Rose procedure. The major disadvantage of the two-dose test, however, was that because of the early interruption of the test, only the height of the blood sugar curve was expressed and not the

more important two-hour value.

The third type of GTT, in vogue at present, involves the oral administration of a single dose of glucose to a fasting individual in the morning and the estimation of blood sugar levels before and at regular intervals after glucose ingestion for a period of two to three hours. The relative advantages of the oral and intravenous tests have been discussed by several authors (Goldberg and Luft, 1948; McIntyre, 1967; Holdsworth, 1969) and it is clear that the objections to the first two tests do not exist here. The single-dose oral GTT is the method of choice in most centres today and it is to this test and its analysis that the remainder of this discussion and the studies contained in this thesis are confined.

The response to a single dose of ingested glucose was documented in 1913 by Jäcobsen. Using a 100 g load and Bang's method for the determination of glucose (Bang, 1913), he demonstrated in normal subjects an appreciable rise in blood sugar within 5 min of glucose ingestion; the maximum level was usually attained in about 30 min, and a return to pre-ingestion levels occurred within an hour and three-quarters. He tested several diabetics with 50 g of bread and showed that the actual rise of the blood sugar was not great but that the fall was slow. Subsequently the changes in blood sugar levels after oral glucose administration were used widely by clinicians in experimental studies in animals and man as an index of the efficiency of blood sugar regulatory mecahnisms. In 1923 Foster (1923b) was able to report

> "The changes in blood sugar following glucose ingestion in normal man are well established. The sharp rise to a maximum followed by a rather rapid return to normal and often markedly hypoglycaemic level has been shown by Jäcobsen and many others".

The shape of the glucose tolerance curve was influenced by the magnitude of the administered glucose load which was either a standard amount of 25 g (Rabinowitch, 1927), 50 g (Friedenson et al., 1928; Cavett and

Seljeskog, 1933; Himsworth, 1935; Somogyi, 1950) or 100 g (Janney and Isaacson, 1918; Foster, 1923a; Mosenthal and Barry, 1946; Somogyi, 1948) or an amount proportional to body weight ranging from 1.0 g/kg (Hagedorn, 1921; Holst, 1922; Enocksson, 1932) to 1.75 g/kg (Hamman and Hirschmann, 1917; Glassberg, 1930). In general, larger doses of glucose increased the duration but not the height of the hyperglycaemic response (MacLean and de Wesselow, 1921; Hansen, 1923; Tisdall et al., 1925) and resulted in a curve which often showed a short hypoglycaemic phase (Peters and Van Slyke, 1946). MacLean and de Wesselow (1921) studied the response to glucose loads ranging from 5-100 g; they found no difference in the peak glucose level with loads between 25-100 g but the duration of the hyperglycaemia was slightly prolonged with the higher doses. Similarly, Peters and Van Slyke (1946) showed that increasing the dose of dextrose from 1.0-1.5 g/kg may delay by as much as an hour the return of glucose levels to fasting values. Many of the early studies of glucose tolerance were reviewed by MacLean and de Wesselow (1921) and Hale-White and Payne (1926).

Little work has been done on the physiological mechanisms responsible for the shape of the oral glucose tolerance curve. Two of the factors which have been accorded great importance are the rate of gastric emptying and the rate of intestinal absorption of glucose. In 1919 Bailey wrote

> "An important observation of all of these tests is the marked disproportion between the greatest blood sugar concentration and the amount of sugar ingested, allowances being made for differences in body weight. This disproportion is seen in the work of individual authors and makes one think that sugar tolerance tests based on blood sugar estimations are really tests of intestinal absorption".

Hale-White and Payne (1926), however, pointed out that the rate of gastric emptying seemed to have no effect on the ascending limb of the curve as this was similar in subjects given glucose orally and in three subjects given glucose directly into the duodenum. They also showed that the

blood sugar would not only start falling but return to normal while a considerable amount of glucose was still present in the stomach. Foster (1923b) commented

> "Obviously the rise and fall are dependent on the relative velocities of the inflow of glucose from the intestine and its subsequent removal from the blood stream by the tissues. It is not surprising that when a large amount of readily diffusible glucose is suddenly introduced into the alimentary tract, the rate of absorption should exceed the rate at which the tissues can abstract it from the blood. But it is not so clear why the curve should again fall to normal as rapidly as it does at a time when the rate of absorption from the gut can scarcely be diminished. Nor is it clear why the curve should fall below the normal fasting level subsequently".

Hypoglycaemia after the ingestion of glucose was demonstrated by Liefmann and Stern in 1906. In 1910 Frank suggested that this hypoglycaemia was due to overstimulation of the glycogen forming function of the liver. MacLean and de Wesselow (1921) put forward the same hypothesis and presented a comparison of the blood sugar curves of a normal man and of a severe diabetic after ingesting 50 g glucose. The curves both rose sharply by nearly the same amount for the first half hour. At this point the normal curve broke and soon began to fall rapidly to the fasting level while the curve of the diabetic continued to rise for two hours or more i.e. "presumably as long as absorption continued". These authors (MacLean and de Wesselow, 1921) concluded that apparently, in the normal, some mechanism which was dormant for the first half hour had been awakened and intervened to check the rising hyperglycaemia; they argued that the presence of an excess of sugar in the circulation stimulated the mechanism which deals with sugar and assumed it to be glycogen synthesis largely in the liver. The period of hypoglycaemia in the normal curve they proposed was due to an "overactivity of the awakened glycogen-forming function. In the severe diabetic glycogen synthesis does not occur appreciably and the hyperglycaemia is excessive" .

The concept of a mechanism responsible for glucose disposal remaining

dormant for 30 min or so after glucose ingestion was wholly consistent with the studies of Bornstein and Holm (1922). In 1922 these workers found during 100 g GTTs that whereas the blood sugar began to rise immediately after glucose ingestion, the rise in respiratory quotient exhibited a definite latent period of 30-60 min; that is, a rapid increase in sugar oxidation began at about the same time as the break in the blood sugar curve which was believed to be due to glucogen synthesis. After it had once begun the increased rate of sugar oxidation persisted for several hours, during which time the blood sugar might have fallen to normal or low levels. There was no parallelism between the blood sugar concentration and the rate of sugar oxidation; on the contrary, the relationship seemed to be inversed.

Cori and Cori, in a series of studies (Cori, 1925; Cori, 1926a,b; Cori and Cori, 1926; Cori and Cori, 1927a,b,c) described in greater detail the factors concerned in the control of glucose homeostasis in the fasting state and the mechanisms which determine the form of the alimentary blood sugar curve under different conditions. They suggest that at all times glucose is being absorbed from the blood by the muscles and other tissues to be stored as glycogen, which in turn, is continually decomposed to lactic acid and oxidised. The rate of removal in the resting, fasting state, however, is too small to be readily detected. The blood sugar is as continually replenished from the hepatic stores of glycogen which are obtained from ingested preformed glucose or by the transformation in the liver of other carbohydrates or protein. The initial rise of arterial and venous blood sugar after the ingestion of glucose is an expression of the absorption of sugar from the alimentary tract. The subsequent reduction of the blood sugar to the normal level is the result of two factors: (1) the removal of sugar by the liver to form glycogen and (2) the removal of sugar by the tissues and especially muscle, for the formation of tissue glycogen and for combustion. It

is the latter process that is evidenced in the arterio-venous (A-V) difference. Insulin appears to exert its chief effect in promoting removal and consumption of sugar by the tissues. Because the blood sugar begins to fall while absorption of carbohydrate is at its height and because alimentary hyperglycaemia is eventually succeeded by a period of hyperglycaemia, it may be inferred that the mechanisms for the removal of sugar from the blood and its disposal have a certain inertia. They are activated to their full extent only after the blood sugar has risen measurably above the normal level and are not again checked until it has fallen distinctly below this level. It is clear from the foregoing how accurate and detailed a grasp of the field these workers had.

Although the role of the liver in handling absorbed glucose had been emphasised since the early work of Bernard and Pavy, the importance of the extrahepatic or peripheral tissues (mainly muscle) in glucose disposal was not fully appreciated until large A-V differences were demonstrated across the forearm after oral glucose loading. The fact that the concentration of glucose in systemic arterial blood exceeds that in systemic venous blood was first demonstrated by Chaveau in 1856. He conducted a careful study of sugar concentrations in a variety of vessels in animals and found that under conditions of fasting or meat feeding the concentration of sugar was always higher in the hepatic veins than in any other vessel including the portal vein. Chaveau (1856) also found that the concentration of sugar was the same on the two sides of the heart and in all the arteries, but there was constantly more glucose present in arterial blood than in venous blood. The existence of a difference in the sugar content of arterial and venous blood, greater during digestion, was confirmed by Bernard (1877), Otto (1885) and Pavy (1894). Bang (1913) indicated that he was aware of the A-V difference but despite this, there is little evidence that subsequent workers shared this awareness until Hagedorn published his investigations

in 1920. Indeed, Folin and Berglund (1922) offered as an explanation of the greater alimentary hyperglycaemia found by European workers, the theory that puncture of the finger or ear caused greater pain than venous puncture and, therefore, greater nervousness. After the publication of Hagedorn's studies (Hagedorn, 1920) Scandinavian investigators, at least, began to appreciate the importance of distinguishing between cutaneous and venous blood. It was not until 1923, however, that the distinction was recognised in America when Foster (1923a) confirmed in every respect the observations of Chaveau (1856) and Hagedorn (1920).

It was demonstrated at this time that the sugar concentrations of arterial and capillary blood were identical; the initial data were reported by Hagedorn (1920) and Foster (1923a) and their findings subsequently supported by those of several other workers (Blitstein, 1933; Jonas, 1933; Langner and Fies, 1942; Somogyi, 1948). Foster (1923a), for example showed in a dog that the blood from a paw prick and arterial blood gave readings which were identical within the range of experimental error. Langner and Fies (1942), on the other hand, used blood obtained simultaneously from the radial artery and the finger of human subjects.

During the third decade several workers documented the changes in both arterial and venous glucose levels after glucose loading in man (Holst, 1922; Lawrence, 1924; Cori, 1925; Lundsgaard and Holboll, 1925; Gilbert et al., 1926; Rabinowitch, 1927; Friedenson et al., 1928). The findings were summarised by Friedenson et al. (1928) who wrote

> "All workers agree that in the postabsorptive state the concentrations of sugar in arterial and venous blood are identical. After the ingestion of carbohydrate both rise. The rise of the venous curve is checked before that of the arterial and falls more rapidly. At a variable period after the meal the venous sugar sinks not only to, but actually below, the initial postabsorptive level. This is the postalimentary hypoglycaemia, first described by Bang. The cutaneous

> curve reaches the initial level more slowly than does the venous curve but, like the latter, eventually tends to fall slightly below it. The two curves then converge at the fasting level".

Foster (1923a) emphasised, however, that "the hypoglycaemia which nearly always follows the rise is much more marked in the venous than finger blood - in fact it is often missing in the latter". Friedenson et al. (1928) carried out their studies during 50 g GTTs and noted the greatest A-V differences in the first hour. The A-V difference was usually maximal at the point of greatest hyperglycaemia but then fell progressively as the blood sugar level declined, the arterial and venous curves converging during their return to fasting levels. Foster (1923a) found no definite correlation between the magnitude of the A-V differences and the degree of hyperglycaemia; in contrast, Holst (1922) concluded that such a correlation did exist.

In diabetics Holst (1922), Lawrence (1924), Lundsgaard and Holboll (1928), Rabinowitch (1927) and Glassberg (1930) all found that the alimentary A-V difference was absent or greatly diminished; indeed, Lawrence (1924) reported that venous levels occasionally exceeded those in arterial blood. Both Lawrence (1924) and Lundsgaard and Holboll (1928) claimed that in diabetics reduced A-V differences could be restored to the normal range by the administration of insulin; from this Lawrence (1924) concluded "that, whatever part of the liver and other organs play, insulin acts largely in the muscles or general tissues, or both", and Rabinowitch (1927) suggested that "the magnitude of the A-V difference following glucose is a function of the severity of the disease". Some workers, however, found A-V differences after glucose loading in diabetics to be within the normal range (Glassberg, 1930; Cavett and Seljeskog, 1933) while Somogyi (1938) concluded that most diabetics exhibited A-V values as great as, and often greater than those observed in the average normal person.

In normal subjects Lawrence (1924) reported A-V differences of 20-40 mg/100 ml after the ingestion of 100-200 g glucose while the maximum difference found by Friedenson et al. (1928) after a 50 g load was 50 mg/100 ml. Subsequent authors (Blitstein, 1933; Cavett and Seljeskog, 1933; Jonas, 1933; Marble et al., 1939; Perley and Kipnis, 1967) confirmed, in general, the findings of these earlier workers in terms of both the magnitude of the A-V differences observed and the interrelationships of the arterial and venous glucose curves. The detailed studies of Somogyi (1948), however, merit individual comment. In contrast to previous studies, Somogyi (1948) was able to show that the average A-V difference in the postabsorptive state was not zero, but 4.8 mg/100 ml - indeed the values ranged between 1-7 mg/100 ml in 93% of subjects and up to 13 mg/100 ml in the remainder. Furthermore, he confirmed the identity of glucose levels in arterial and capillary blood. He noted the prompt and steep rise in the A-V difference as seen as the blood sugar began to rise and its somewhat less consistent decline as the hyperglycaemia subsided. He observed that the maximum A-V difference was attained 30-60 min after glucose ingestion and that the increase in the A-V differences was a function of the hyperglycaemic level i.e. the higher the hyperglycaemic peak, the greater the A-V difference. The change in the A-V difference, however, showed a discernible lag compared with the changes in the blood sugar curves - i.e. the A-V difference continued to increase during the second half hour of the GTT when the blood sugar was already falling. The average maximum A-V difference at 60 min after the ingestion of 100 g glucose was 33.5 mg/100 ml, the highest value being 58.0 mg/100 ml; during 50 g GTTs peak A-V differences at 30 or 60 min were smaller varying between 15-30 mg/100 ml (Somogyi, 1950).

During 100 g tests other workers observed even larger A-V differences; for example, a maximum value of 102 mg/100 ml was reported

by Mosenthal and Barry (1946) in a diabetic 30 min after glucose ingestion and in a subsequent study (Mosenthal and Barry, 1962) in normal subjects the highest value observed was 124 mg/100 ml 60 min after glucose loading. Mosenthal and Barry (1950) observed quite commonly that when the venous blood sugar curve was flat, the arterial curve was distinctly elevated. These workers concluded therefore that the arterial curve indicated more accurately than the venous curve glucose absorption from the intestine; the venous curve, on the other hand, appeared to be a more accurate measure of the tolerance to glucose since the A-V difference represented an index of glucose assimilation by peripheral tissues. Even the arterial curve however, may fail to reflect glucose absorption because of "the possibility that liver takes up the glucose passing through it in the portal vein, a factor that might prevent an elevation of arterial blood sugar".

Mosenthal and Barry (1950) calculated that an A-V blood sugar difference of 1 mg/100 ml indicated the disposition of about 1 g of glucose/hour in the muscle and skin. On the basis of the latter and other assumptions, they suggest that 60 g of a 100 g oral load would be taken up by peripheral tissues in two hours; it was emphasised that this was a conservative estimate since the subjects were far from being at rest in the physiological sense and the flow of blood circulating through the extremities was in all probability much greater than allowed for in the assumptions. In support of their estimates, Mosenthal and Barry (1950) quoted the earlier work of Carpenter and Fox (1930). The latter concluded in 1930 that after ingestion, 100 g glucose undergoes combustion at the rate of 10 g/hour and that since hepatic glucose formation is checked by the ensuing hyperglycaemia, there would be in the average normal individual 80 g glucose disposed of by extrahepatic tissues (i.e. 20 g by combustion, 60 g taken up by the peripheral tissues). Mosenthal and Barry (1950) considered that the findings of Carpenter

and Fox (1930) and their own negated the contention of Soskin et al. (1938) that the liver takes care of all the ingested load. Conn and Newburg (1937) calculated on the basis of indirect calorimetry, that during 100 g GTTs in normal subjects, 40-50 g glucose was disposed of by oxidation; they suggested, therefore, that 50-60 g was disposed of by the disposition of glycogen in the liver (and possibly to some extent in the muscles). Somogyi (1948) felt in this respect that the extrahepatic tissues played at least as significant a role as the liver.

The importance of the liver in controlling glucose homeostasis was emphasised by the excellent and meticulous studies of Soskin and Levine and their colleagues (Soskin and Levine, 1952). Earlier Cori (1931) and Staub (1927) concluded that the production of the normal glucose tolerance curve depended on the stimulation of the pancreas by the ingested sugar. The consequent secretion of insulin was supposed to dispose of the incoming sugar by increasing the rates of storage and "oxidation" of the carbohydrate (Staub, 1927; Cori, 1931). The abnormal type of curve characteristic of the depancreatised animal and of the diabetic human was attributed to a lack of pancreatic response, with a consequent inability to dispose of the incoming sugar at the normal rate (Staub, 1927; Cori, 1931). This explanation ignored the vital role of the liver in supplying sugar and the possibility that regulation might also be accomplished by controlling this supply. It was at testing the validity of this explanation that the studies of Soskin, Levine and their colleagues (Soskin and Levine, 1952) were aimed.

In 1934 Soskin et al. (1934) substituted a constant-injection pump for the pancreas as the source of insulin in dogs. Completely depancreatised dogs received constant intravenous injections of insulin at rates just sufficient to maintain a normal constant blood-sugar level in each particular animal. They were therefore restored to normal in a restricted experimental sense except that they could not mobilise

additional insulin. Since, despite this, these animals exhibited perfectly normal tolerance curves, it was concluded that, provided sufficient insulin was present to maintain a constant blood sugar level, no additional secretion was necessary for adequate regulation.

These results directed attention towards the liver as the factor that varied in regulation. Normal dogs were hepatectomised and a constant injection of glucose just sufficient to maintain a normal, constant blood sugar level was substituted for the liver. Since the pancreas remained intact, this type of animal preparation was able to mobilise insulin as required but could not alter the rate at which sugar was being delivered to the blood from the artificial liver. Such animals invariably yielded markedly "diabetic" tolerance curves. It was apparent, therefore, that the normal pancreas was not essential to the regulatory mechanisms responsible for the normal glucose tolerance curve, while the presence of the normal liver was essential. This was consistent with the findings of Weil and Besser (1932) who showed that surgical removal of 50% of the dog's liver caused a delay in clearing the blood of extra glucose.

The homeostatic regulatory mechanism of the liver for the control of the blood sugar level was later subjected to direct proof (Soskin et al., 1938). By correlating the rate of blood flow through the liver of experimental animals (thermostromuhr measurements) with the difference in the sugar content between the blood flowing into and out of this organ, Soskin and his colleagues (1938) calculated the absolute amounts of sugar entering and leaving the liver per unit time during a GTT. It was shown that the liver which released sugar into the blood prior to the administration of glucose ceased to do so almost immediately after glucose loading and, instead, began to take up large quantities of sugar. Following this period of sugar retention, the liver neither took up nor released sugar for about an hour and during this phase the blood

sugar concentration fell below the pre-test level. Thereafter, the liver again began releasing sugar into the blood in normal amounts and blood sugar levels rose not only to, but above the pre-test level. Soskin et al. (1938) concluded that in the liver inhibition of glucose output is a phenomenon separate from that of glucose storage - a conclusion which was wholly confirmed some thirty years later (Mebane et al., 1961; Bishop et al., 1965; Steele et al., 1968; Madison, 1969). Bondy et al. (1949a,b) subsequently obtained essentially similar results in man, using the liver catheterisation technique and the bromsulphthalein method for the estimation of hepatic blood flow.

Although Soskin et al. (1934) had emphasised the hepatic role in blood sugar regulation, Soskin and Levine (1952) cautioned

> "It is not to be supposed, however, that the hepatic mechanism is the only one involved. Glycogen deposition in both the liver and muscle and an increased utilisation of sugar by the extrahepatic tissues undoubtedly play their parts. These processes, like hepatic homeostasis, are under the influence of the blood sugar level".

Soskin and Levine (1952) noted that Cori and Cori (1929) "have pointed out that the rate of glycogen deposition depends upon the concentration of sugar in the blood". Soskin and Levine (1952) recalled that previously they themselves (Soskin and Levine, 1937)

> "have shown that the rate of sugar utilisation by the extrahepatic tissues varies directly with the height of the blood sugar level. It seems logical to assume that smaller amounts of sugar, especially if they enter the circulation via the portal vein, may be fully compensated for by hepatic inhibition alone. Larger amounts of sugar will invoke hepatic storage as well. Still larger amounts, which, in spite of the foregoing, raise the systemic blood sugar level, will bring into play the additional factors of extrahepatic storage and increased utilisation".

A major criticism of all the studies of A-V differences quoted above, with the exception of those by Soskin et al. (1938) and Bondy et al. (1949a,b), is the assumption inherent in them that A-V differences alone reflect tissue uptake and that in the calculation of the latter the level of regional blood flow may be ignored. As noted

above, Rabinowitch (1927) found reduced A-V differences in diabetics during GTTs and suggested that "the magnitude of the A-V differences following glucose is a function of the severity of the disease". The implication that A-V differences more or less reflected tissue uptake was accepted. Somogyi (1949) refers to blood flow briefly only, in one of his papers on A-V differences. Lang **et al.** (1954) studied peripheral glucose uptake in anaesthetised dogs. They measured blood flow to the legs of these animals but only for 15 min before commencing the main glucose observations which continued for 60-120 min thereafter; blood flow varied up to twofold in individual dogs. Bondy in a further study (Bondy, 1952) took pains to measure hepatic blood flow when using the differences between sugar concentrations in arterial and hepatic venous samples in order to calculate hepatic glucose output. Yet later in 1956, Bondy and Cardillo accepted peripheral A-V differences alone as indications of peripheral glucose uptake. In discussing their results they stated "The validity of the A-V difference as an index of carbohydrate utilisation is well established" and in support of this statement quoted the studies of Rabinowitch (1927), Somogyi (1948) and Lang et al. (1954). Bondy and Cardillo (1956) concluded "The A-V differences measured in these experiments are, therefore, direct reflections of peripheral utilisation".

Andres et al. (1956) demonstrated their awareness of the absolute requirements for the valid application of the Fick principle in the calculation of tissue glucose uptake. The lack of attention that had been paid to the determination of blood flow in this context was further emphasised in 1959 by Butterfield and Holling. It is only in the past 16 years that meaningful studies of peripheral glucose uptake in man have been carried out and it is these studies (referred to in subsequent chapters) and their relevance to the interpretation of the glucose tolerance curve which have proved the inspiration for this thesis.

CHAPTER 4

SUBJECTS AND METHODS

(a) SUBJECTS

Detailed clinical data of the various groups are given below in the relevant chapters. Suffice it to note here that all subjects studied were nonobese i.e. less than 120% of ideal body weight (Metropolitan Life Insurance Tables, 1959) and all remained fully ambulant throughout the periods of dietary preparation prior to the performance of the GTTs (Blotner, 1945; Lipman et al., 1970, 1972).

(b) COMPOSITION OF THE DIETS

(1) Normal carbohydrate diet

This diet contained sufficient calories to maintain a constant body weight (determined from the dietary history of each subject) and supplied a minimum of 220 g carbohydrate daily (Conn, 1940). The diet on the average had the following composition:- 45% carbohydrate; 40% fat; 15% protein.

(2) Low carbohydrate, low calorie diet

This diet provided 28.3 g carbohydrate, 99.2 g fat and 6.1 g protein per day.

(c) EXPERIMENTAL CONDITIONS

The GTT (100 g glucose dissolved in 400 ml water and ingested over a period of 2 min) was carried out during the morning following an overnight fast in a quiet room in which the air temperature was maintained between 21-24^{o}C. The volunteers remained recumbent throughout the GTT and had rested in this position for at least 60 min before basal observations were made. Forearm blood flow was monitored and samples

of arterialised venous (AV) and mixed venous (MV) blood were drawn basally and at 15 min intervals for 180 min after glucose administration, as outlined below. Blood was sampled over a 30-60 second period.

MV blood (see section (e) below) was drawn from a large antecubital vein receiving both superficial and deep branches but which appeared to drain predominantly the deep compartment of the forearm; this vein, when occluded proximally, could not be seen or palpated for more than 2-3 cm distal to the antecubital crease. Blood was withdrawn without venous occlusion through an indwelling plastic cannula (Braunula No.1, Armour Pharmaceutical Company Ltd., Eastbourne, Sussex, England) placed 1-2 cm distal to the antecubital crease. Patency of the cannula was maintained between sampling by means of a well-fitting stylet previously moistened with a 50% sodium citrate solution.

AV blood (see section (d) below), sampled from the contralateral hand, was used throughout this study as a substitute for arterial blood (Mottram and Butterfield, 1961), and was drawn through an indwelling needle (Butterfly-21 infusion set, Abbott Laboratories, North Chicago, Illinois, U.S.A.) inserted in a retrograde direction and placed as distally as possible in a vein on the posterior aspect of the hand. Patency of this needle was preserved with a constant saline infusion (1.0 ml/min). Before collecting samples at this site, between 1-2 ml blood contaminated with saline were withdrawn and discarded, the latter representing a volume 2-4 fold that of the entire infusion set. For 20-30 min prior to glucose administration and for the entire duration of the GTT (including the sampling periods), the hand remained enclosed between two heating pads warmed to 40-50^{o}C.

Forearm blood flow (see section (f) below) was determined in the arm bearing the MV cannula by venous-occlusion plethysmography with a collecting pressure of 60 mm Hg and a wrist occlusion pressure of 220 mm Hg,

the wrist cuff serving to prevent contamination of venous samples with arterialised blood from superficial hand veins. Changes in forearm volume were measured by means of a temperature compensated mercury-in-rubber strain gauge placed at the junction of the upper third and lower two-thirds of the forearm. The limb was positioned horizonatally at heart level and at an angle of 20-30° from the body. The wrist cuff (width 5 cm) was inflated 4 min before sampling and beginning one minute later, blood flow was recorded for 8-10 seconds out of every 15 seconds during the 3 min immediately prior to collection of the blood samples. Samples of AV and MV blood were drawn simultaneously and immediately following the completion of the last inflow recording. Inflation of the wrist arresting cuff, but not the collecting cuff, was maintained during sampling (Loughlin et al., 1943). During blood flow determinations, the hand was kept motionless in order to avoid fluctuations in forearm blood flow. Calibration of the gauge was carried out by stretching the latter by a known extent after removal from the arm. Plethysmograms were recorded on a Type M8 Recorder (Devices, Herts., England). The experimental technique for the study of forearm metabolism is illustrated in Figs. 1-4.

Forearm glucose uptake (FGU) was calculated as the product of the mean plasma flow from each set of recordings and the difference in plasma glucose concentrations between the corresponding samples of AV and MV blood. It has been pointed out (Butterfield et al., 1963) that, when calculated in this way, FGU refers to tissue rather than cellular glucose uptake. Haematocrit determinations by the microhaematocrit method were made on MV blood collected prior to and at half-hourly intervals after glucose loading and the mean used in the calculation of forearm plasma flow. At least two complete sets of basal observations were made before glucose loading and all values for basal metabolism have been expressed as their means. In separate experiments designed to

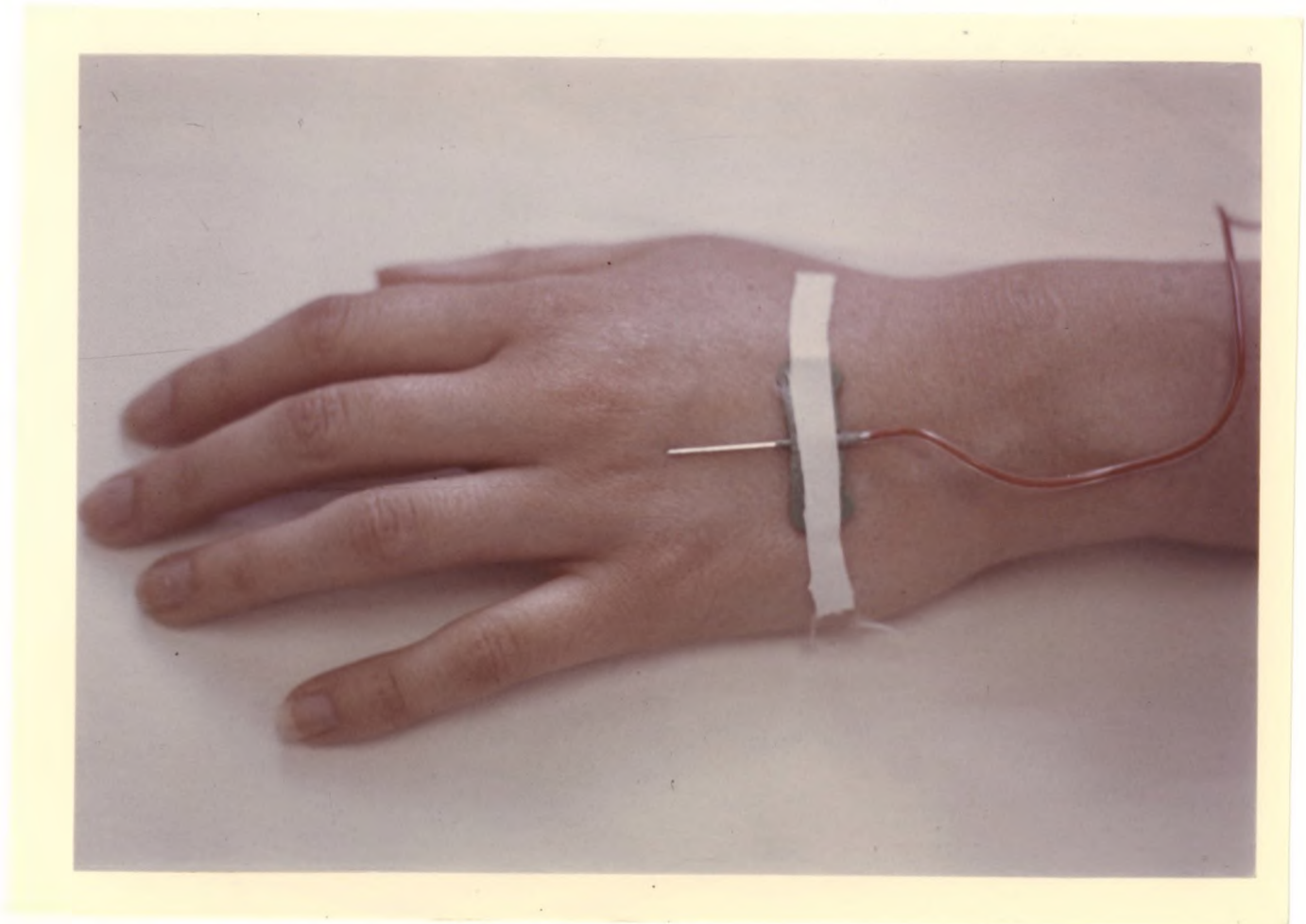

FIG. 1 Indwelling needle of a Butterfly Infusion Set (no. 21)

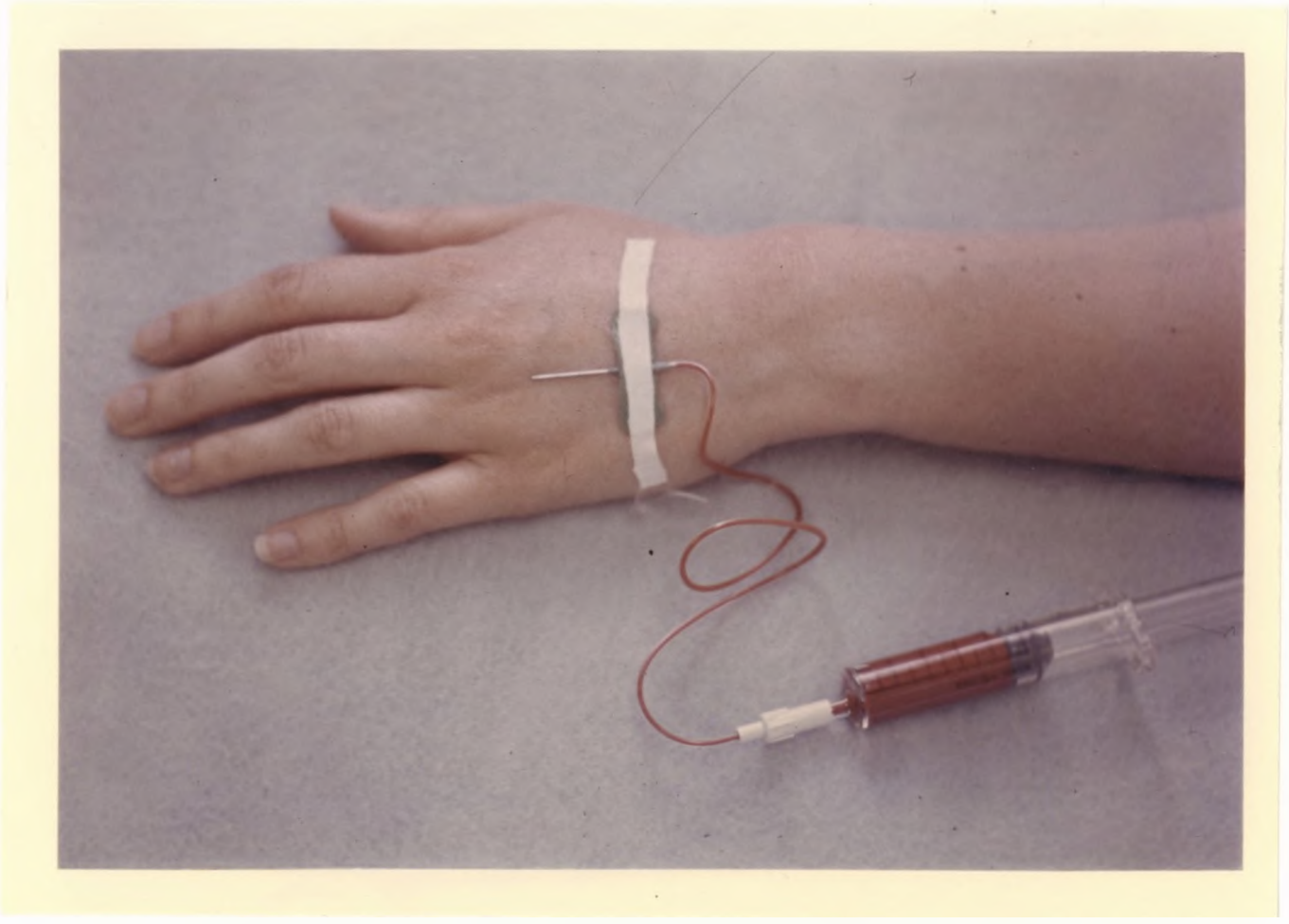

FIG. 2 Sampling of arterialised venous blood

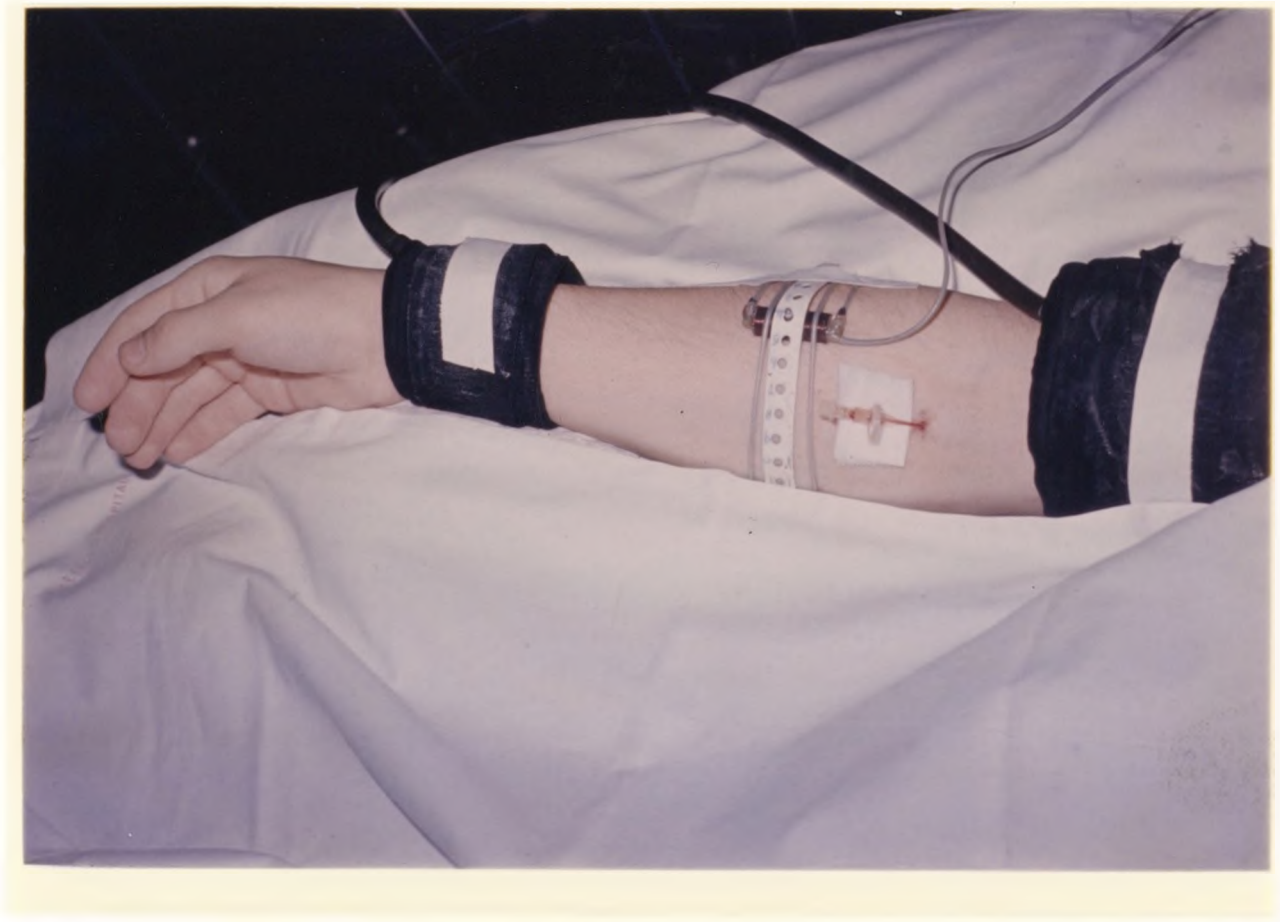

FIG. 3a Forearm bearing the wrist cuff, the collecting cuff,

the indwelling mixed venous cannula and the strain gauge

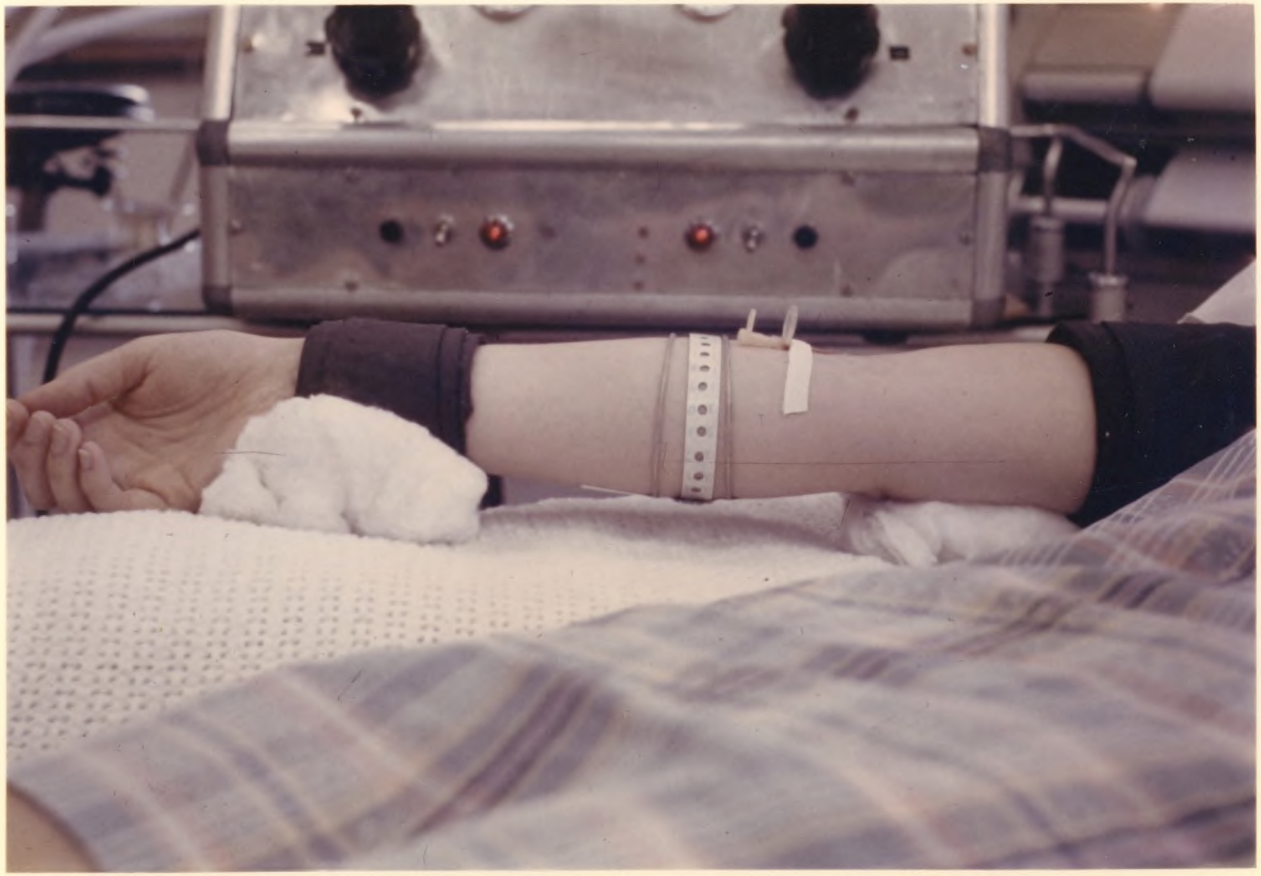

FIG. 3b Forearm bearing the wrist cuff, the collecting cuff,

the indwelling mixed venous cannula and the strain gauge

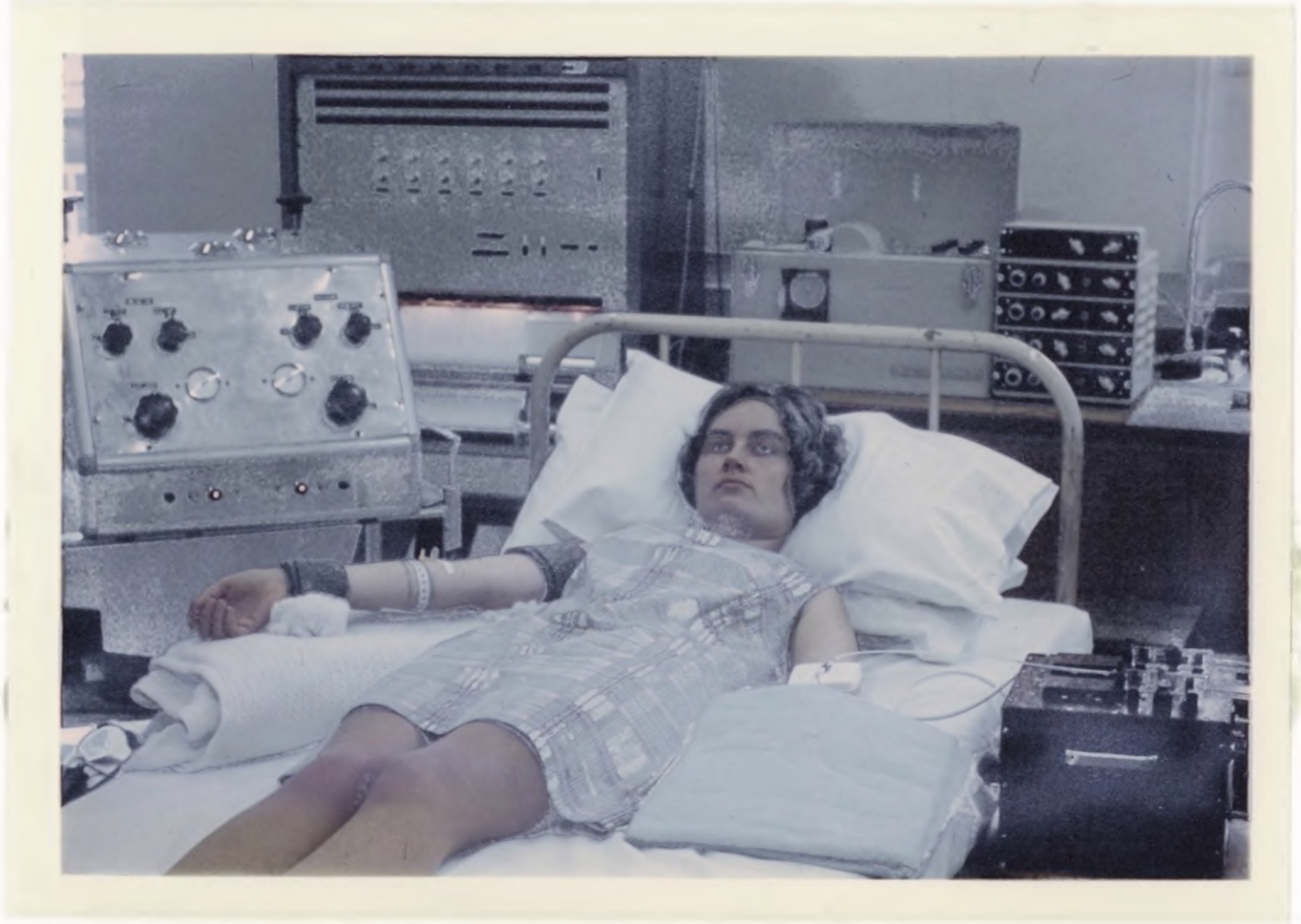

FIG. 4 Normal subject during a typical experiment

validate the use of AV blood and in the insulin infusion experiments (see below, Chapter 6), the brachial artery was punctured just proximal to the antecubital fossa using a Cournand needle; a polyethylene catheter was passed through the needle for 8-10 cm by the Seldinger technique. During the insulin infusion experiments insulin was infused into the brachial artery at the rate of 100 μU/kg/min for 30 min (Zierler and Rabinowitz, 1963). Forearm volume between the proximal border of the wrist arresting cuff and the antecubital crease, was determined by water displacement.

Because the two forearms of the same individual may differ significantly in terms of both tissue mass and patterns of venous drainage, it could not be assumed that the results of an experiment on one arm would necessarily provide a valid control for a second investigation carried out on the contralateral arm. Thus a routine practice was adopted from the outset whereby, in each experiment on the same subject, the same antecubital vein on the same forearm was cannulated at the same point. Accordingly, it was ensured that during each GTT the response of the identical tissues in each individual were being compared before and after the low-carbohydrate diet.

In each of three subjects the half-time disappearance of exogenous insulin from serum was determined before and after 3-4 days on a low carbohydrate diet. Following an overnight fast, samples of antecubital venous blood were drawn through an indwelling cannula for the estimation of basal insulin concentrations. The decrease in serum insulin levels was then followed after the intravenous injection of insulin (Boots, England) in the contralateral arm in a dose of 0.1 units/per kg body weight. The half-time disappearance was determined by plotting insulin levels logarithmically against time and extrapolating from the line of best fit.

(d) USE OF ARTERIALISED VENOUS (AV) BLOOD

Because direct arterial puncture inevitably provokes anxiety in a proportion of subjects and because the validity of the data was dependent on the complete and continued absence of heightened sympathetic activity (Porte, 1969; Somogyi, 1950) in each volunteer, AV blood was sampled as a substitute for arterial blood from the outset. I reasoned that the errors resulting from the present technique would be small (Mottram and Butterfield, 1961) and in any event, considerably less than those which might be anticipated in association with repeated arterial catheterisation. The technique of sampling AV blood is simple and painless and consequently ensured in all volunteers the ready maintenance of a basal emotional state throughout each experiment. The justification for adopting this technique was provided by the results of separate experiments in which samples of arterial and AV blood were simultaneously drawn at varying intervals following an oral glucose load. These data (Table 1) verified that the arterio-AV (A-AV) glucose differences were small and unrelated to the corresponding arterial concentration and that they remained fairly constant over the range of values observed during the GTTs; this applied equally to the A-AV differences for insulin (Table 2) and lactate (Table 3), all of which are summarised in Table 4. A comparison of these results (Table 4) with the mean AV-MV differences observed during the GTTs (Table 5) indicates that any errors arising from the use of AV rather than arterial blood will be small, indeed negligible, except in the determination of basal FGU. The latter is supported by the remarkable similarity between the AV-MV differences shown in Table 5 and the capillary-venous differences reported by Somogyi (1948) during 100 g GTTs. Figs. 5 and 6 illustrate the results of two of these experiments (See Appendix, Tables 1 and 2) in which MV blood was drawn in addition to arterial and AV blood. That the change in ketone body

TABLE 1

Comparison of plasma glucose concentrations (mg/100 ml) in arterial (A) and arterialised venous (AV) blood following 100 g oral glucose load

Time	J.M.			M.B.			T.P.		
min	A	AV	A-AV	A	AV	A-AV	A	AV	A-AV
0	87	84	3	81	77	4	85	86	-1
0	87	86	1	80	78	2	85	84	1
15	121	118	3	136	134	2	-	-	-
30	169	163	6	185	184	1	164	154	10
45	191	186	5	140	138	2	178	172	6
60	171	172	-1	126	125	1	193	193	0
75	145	150	5	118	119	-1	175	173	2
90	128	129	-1	129	125	4	172	171	1
105	112	112	0	126	125	1	164	169	-5
120	103	104	-1	115	113	2	138	143	-5
135	105	106	-1	94	94	0	140	146	-6
150	104	105	-1	66	66	0	148	154	-6
165	95	97	-2	57	57	0	-	-	-
180	72	76	-4	56	55	1	139	146	-7

	T.S			J.H.			R.J.		
	A	AV	A-AV	A	AV	A-AV	A	AV	A-AV
0	88	85	3	100	100	0	100	92	8
20	166	152	14	162	165	-3	133	123	10
40	174	170	4	186	184	2	160	157	3
60	123	119	4	160	162	-2	142	141	1
80	121	115	6	141	144	-3	126	122	4
100	-	-	-	140	141	-1	96	94	2
120	-	-	-	149	144	5	88	87	1

TABLE 2

Comparison of serum insulin concentrations (μU/ml) in arterial (A) and arterialised venous (AV) blood following 100 g oral glucose load

Time	T.S.			J.H.			R.J.		
min	A	AV	A-AV	A	AV	A-AV	A	AV	A-AV
0	12	12	0	27	21	6	7	7	0
20	130	130	0	48	53	-5	42	37	5
40	118	128	-10	69	68	1	65	66	-1
60	50	51	-1	49	53	-4	67	59	8
80	50	55	-5	47	45	2	72	70	2
100	-	-	-	42	39	3	38	40	-2
120	-	-	-	40	37	3	27	26	1

TABLE 3

Comparison of plasma lactate concentrations (mmol/l) in arterial (A) and arterialised venous (AV) blood following 100 g oral glucose load

Time	T.P.			M.B.			J.M.		
min	A	AV	A-AV	A	AV	A-AV	A	AV	A-AV
0	0.73	0.76	-.03	0.47	0.46	.01	0.47	0.47	.0
15	-	-	-	0.45	0.44	.01	0.43	0.45	-.02
30	0.84	0.81	.03	0.54	0.53	.01	0.52	0.52	0
45	0.92	0.90	.02	0.70	0.58	.12	0.58	0.61	-.03
60	0.99	1.03	-.04	0.68	0.65	.03	0.60	0.65	-.05
75	0.87	1.04	-.17	0.57	0.63	-.06	0.58	0.63	-.05
90	0.87	0.90	-.03	0.61	0.59	.02	0.54	0.60	-.06
105	-	-	-	0.62	0.57	.05	0.46	0.49	-.03
120	0.78	0.80	-.02	0.67	0.61	.06	0.43	0.46	-.03
135	0.68	0.66	.02	0.58	0.57	.01	0.39	0.43	-.04
150	0.60	0.62	-.02	0.45	0.47	-.02	0.35	0.40	-.05
165	0.53	0.63	-.10	0.36	0.38	-.02	0.33	0.37	-.04
180	0.58	0.58	0	0.28	0.31	-.03	0.31	0.31	0

TABLE 4

Mean differences between concentrations in arterial (A) and arterialised venous (AV) blood

	Differences A - AV	Subjects tested	Pairs of observations
Plasma glucose mg/100 ml	+ 1.0 ±.0.6*	6	59
Serum insulin μU/ml	+ 0.2 ± 1.0	3	19
Plasma lactate mmol/l	-0.01 ± 0.01	3	37

*Standard error of mean

TABLE 5

Mean arterialised venous - mixed venous differences following 100 g oral glucose load in 25 normal subjects*

		Minutes					
		0	15	30	45	60	75
Glucose mg/100 ml	25	2.0 ± 0**	22 ± 2	35 ± 3	40 ± 3	41 ± 3	35 ± 3
Insulin μU/ml	13	1 ± 1	-	29 ± 5	-	18 ± 5	-
Lactate mmol/l	11	.01 ± .01	-	.16 ± .06	-	.10 ± .04	-

*Data based on results detailed in Table 16, Chapter 5

**Standard error of mean

TABLE 5 Cont.

Mean arterialised venous - mixed venous differences following

100 g oral glucose load in 25 normal subjects

	Minutes						
	90	105	120	135	150	165	180
Glucose mg/100 ml	32 ± 2	29 ± 3	25 ± 2	26 ± 2	24 ± 2	18 ± 2	16 ± 2
Insulin μU/ml	16 ± 4	-	13 ± 6	-	6 ± 2	-	0 ± 1
Lactate mmol/l	.06 ± .05	-	.02 ± .09	-	-.08 ± .02	-	-.11 ± .03

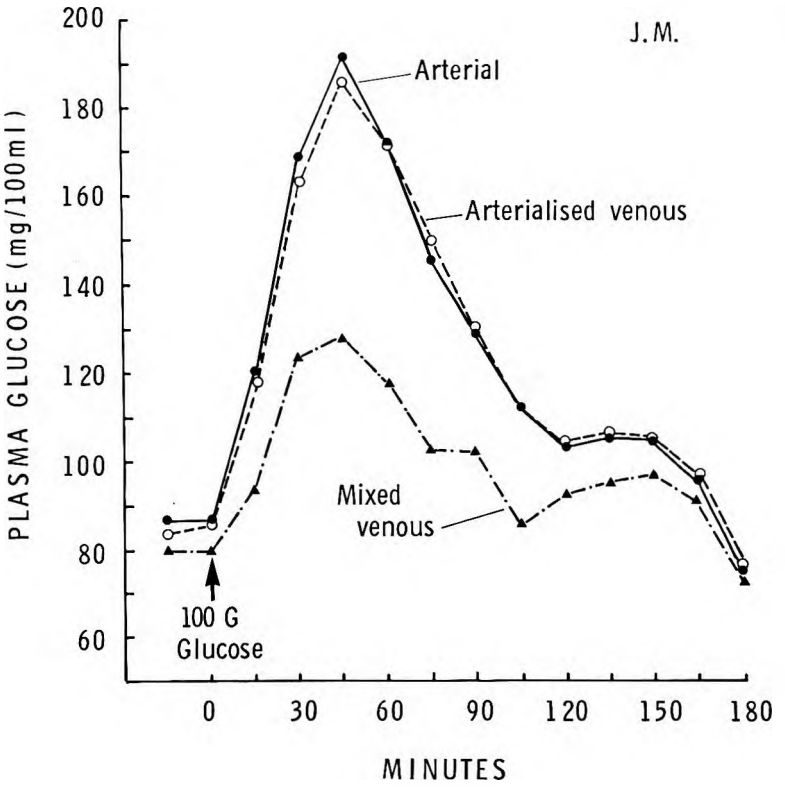

FIG. 5 Comparison of plasma glucose concentrations in arterial, arterialised venous and mixed venous blood following 100 g oral glucose load in a normal subject

concentrations across the forearm is similar whether arterial or AV blood is sampled, as illustrated in Chapter 5 (cf. Figs. 20 and 22). The oxygen saturation (mean ± SEM) of AV blood in 29 separate experiments involving 11 different volunteers was 91.7 ± 0.3% and the necessity for warming the hand in order to achieve full arterialisation of the blood in the hand veins is illustrated by the results shown in Table 6. Mottram and Butterfield (1961) have pointed out that warming the hand may cause a rise in general body temperature and consequently a vasodilatation in the forearm skin vessels; the level and constancy of forearm blood flow throughout the GTTs (see Chapter 5, Table 16; Chapter

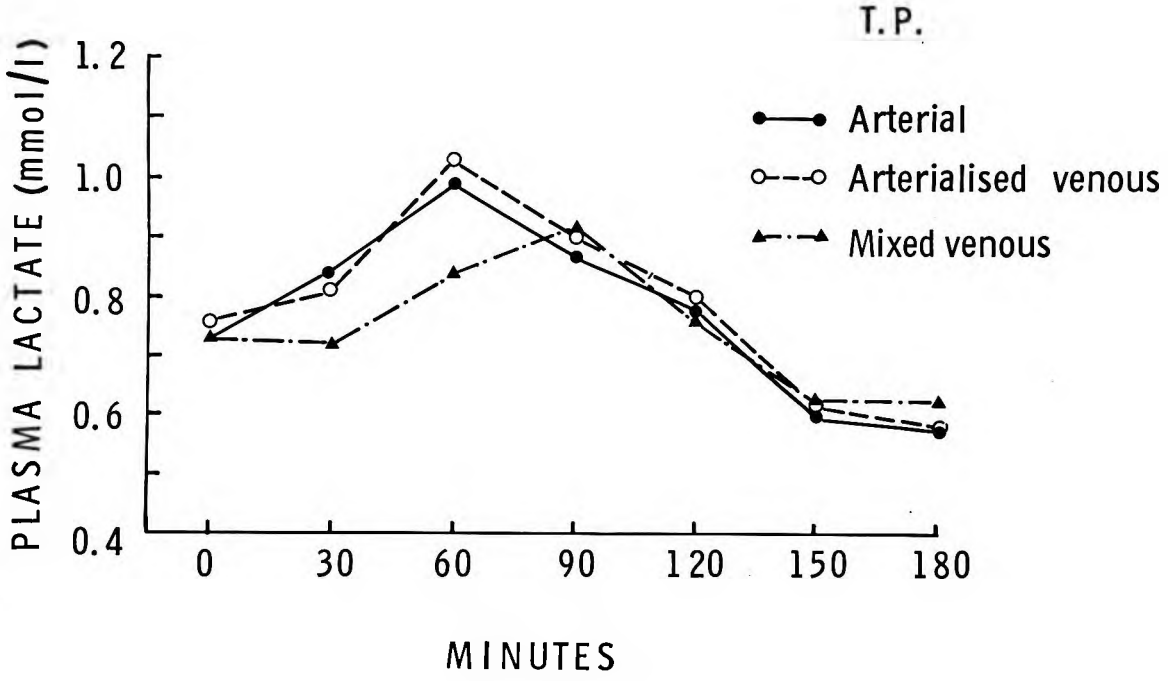

FIG. 6 Comparison of plasma lactate concentrations in arterial, arterialised venous and mixed venous blood following 100 g oral glucose load in a normal subject

6, Tables 22 and 23; Chapter 7, Tables 31 and 32; Chapter 8, Table 39) indicates however, that such reflex vasodilatation could not have been present to any significant degree in our studies.

(e) USE OF MIXED VENOUS (MV) BLOOD

It is questionable whether the greater value attributed to the sampling of deep rather than mixed venous blood for the study of forearm muscle metabolism is in reality anything more than marginal. Although previous workers (Roddie et al., 1956; Coles et al., 1958; Idbohrn and Wahren, 1964) have claimed that the composition of deep venous blood is entirely dependent on the metabolic activity of muscle, there is now evidence to suggest that this is not necessarily so and that in the

TABLE 6

Influence of warming the hand on the composition of arterialised venous blood

Subject		Oxygen saturation	Plasma glucose
		%	mg/100 ml
G.P.	Room temp. (22°C)	64.3	73
	After heating	94.9	87
U.A.	Room temp. (20°C)	87.2	104
	After heating	91.8	108
R.J.	Room temp. (22°C)	93.0	98
	After heating	92.8	103

resting state the deep venous effluent may be no more representative of muscle alone than blood sampled from a carefully selected mixed vein. This is based on the probable underestimation in the past of the influence of two factors, separately or in combination, on the composition of deep venous blood; these are (1) mixing of blood between the superficial and deep venous systems within the forearm and (2) the metabolic activity of the intermuscular adipose tissue.

There are known luxuriant intercommunications between superficial and deep veins in the forearm (Schaeffer, 1953). Venous effluent from the superficial bed (draining mainly adipose tissue) may flow in part through communicating veins to join the deep venous effluent (largely draining muscle, but including intermuscular adipose tissue). Thus a catheter placed in a deep vein of the forearm may sample not only effluent from muscle and an uncertain mass of intermuscular adipose tissue, but also an uncertain contamination by superficial venous blood. The latter would apply particularly in experiments in which blood flow is monitored by a plethysmographic procedure since when a cuff above the elbow is inflated to a pressure of 50-70 mm Hg, blood from superficial veins passes rapidly into the deep vessels mixing with deep venous blood (Mottram and Butterfield, 1961). Moreover, even in the absence of proximal venous occlusion, intermixing of superficial and deep venous effluent may take place within the forearm. Thus having injected Evans Blue into a superficial vein, Christensen and Ørskov (1968) were able to aspirate dye from catheters placed unquestionably deep in the forearm musculature of some normal subjects. Accordingly, unless volunteers are carefully selected for participation in a study on the basis of forearm venous patterns (Christensen and Ørskov, 1968), it cannot be assumed that in all cases arterio-deep venous (A-DV) differences necessarily reflect changes originating exclusively in muscle. Secondly, the metabolic influence of the intermuscular adipose tissue on the

composition of deep venous blood has been emphasised by the recent studies of Zierler et al., (1968). These authors have verified that their previous inability (Baltzan et al., 1962) to demonstrate FFA uptake by forearm muscle using A-DV differences was due to the concomitant delivery of FFA to deep venous blood by the intermuscular adipose tissue. Thus the degree to which A-DV differences are determined by the metabolism of skeletal muscle alone remains unclear and this must be borne in mind in the interpretation of experimental data.

The passage of blood from the superficial to the deep venous bed (Mottram and Butterfield, 1961) is almost certainly the basis for the significant increases in venous blood sugar levels which Loughlin et al. (1943) observed following the application of a venous tourniquet. The latter workers (Loughlin et al., 1943) recommended that for the greatest accuracy a tourniquet should not be used in obtaining blood for venous blood sugar determinations. This recommendation is consistent with the findings of Whichelow et al. (1967) that during 50 g GTTs the sugar concentrations in superficial venous blood remain considerably higher than those in deep venous blood. Accordingly in the present investigations inflation of the venous occlusion cuff was not maintained during blood sampling.

Since the express aim was to study the role of peripheral tissues as a whole in glucose disposal rather than to quantitate the relative contribution of individual peripheral tissues, it followed that total forearm glucose uptake (FGU) should be used as the index of the peripheral tissue response. The determination of FGU is most appropriately given as the product of total forearm blood flow (F_T) and arterio-mixed venous (A-MV) glucose differences, since the latter take into account the responses of both the muscle and adipose tissue within the forearm. For reasons detailed above, the sampling of AV rather than arterial blood

has been preferred and we therefore have

$$FGU = F_T (AV-MV)$$

In view of the markedly different glucose levels which may co-exist in adjacent superficial and deep veins (Holling, 1939; Whichelow et al., 1967), the choice of antecubital vein for sampling (see above) is clearly of the utmost importance.

Although total forearm flow is easily determined by either the dye dilution method or venous occlusion plethysmography (Greenfield et al., 1963; Abramson, 1967), the partition of flow within the forearm is not. This has not proved a disadvantage in the present investigation, however, since in terms of the aims the need for the introduction of a correction factor (Andres et al., 1956) has not arisen.

It is likely that because of the variation in forearm venous patterns (Mottram and Butterfield, 1961) existing between the different volunteers, the antecubital vein chosen for cannulation did not receive blood in identical proportions from the superficial and deep vascular beds in all subjects. The results presented in the succeeding chapters however, represent the means of several different sets of observations and it is reasonable to assume therefore than any errors arising from the latter would cancel each other out in the calculation of the mean responses.

Theoretically, glucose utilisation by erythrocytes in their passage through the forearm could influence the venous glucose and lactate concentrations irrespective of any change in forearm tissue metabolism. In the context of the present experiments, however, the magnitude of such utilisation and its influence on AV-MV glucose and lactate differences may be regarded as negligible and accordingly may be ignored (Cahill and Owen, 1968).

(f) USE OF THE MERCURY-IN-RUBBER STRAIN GAUGE PLETHYSMOGRAPH

Since Hewlett and van Zwaluwenberg (1909) modified the technique of Brodie (1907), venous occlusion plethysmography has been widely employed to estimate blood flow in fingers, toes and limbs. The validity of the venous occlusion technique of plethysmography rests upon four basic assumptions:

(1) that the application of the pneumatic collecting cuff pressure does not affect arterial pressure or inflow

(2) that complete venous tamponade is effected for a finite period

(3) that the resulting increase in venous pressure does not initially reduce the rate of arterial inflow and

(4) that the impounded blood causes the segment under study to swell proportionally to the rate of arterial inflow without significant displacement of tissue or body fluids from the plethysmograph.

Adequate evidence exists which supports the validity of all these assumptions (Hewlett and van Zwaluwenburg, 1909; Wilkins and Bradley, 1946; Formel and Doyle, 1957) and in this regard the good agreement of metered and plethysmographically estimated arterial inflow observed by Formel and Doyle (1957) is particularly gratifying. These latter authors (Formel and Doyle, 1957) concluded that venous occlusion plethysmography permits satisfactory estimation of segmental forearm flow under normal haemodynamic conditions.

The determination of forearm blood flow using the mercury-in-rubber strain gauge has been fully discussed by Abramson (1967) and Greenfield et al., (1963). The attractive features of the strain gauge are its small size and the comparative simplicity of its construction and application; furthermore, its use does not necessitate the enclosure

of the arm in an artificial environment, e.g. water at 34°C, and consequently readings may be obtained under more physiological conditions. An important limitation of the method, however, is that absolute changes in limb volume are not recorded directly but instead are deduced from linear alterations which are presumed to be directly related to the volume change. Quantification of volume change presents a problem because of the lack of a precise and convenient method of calibration. Whitney (1953) calibrated the gauge on the limb involved but subsequent authors (Rushmor,,1955a,b; Holling et al., 1961; Greenfield et al., 1963) have relied on a "physical" calibration which is not carried out on the limb under observation and which involves stretching the whole strain gauge by a known extent after removal from the limb. Although such "physical" calibration may be expected to underestimate absolute volume changes (Greenfield et al., 1963), a direct comparison of blood flow measurements made with the strain gauge and water-filled plethysmograph has shown that in general there is good agreement between the techniques whatever the method of calibration (Whitney, 1955; Clarke and Hellon, 1957; Holling et al., 1961; Paulev, 1969; Dahn and Hallbrook, 1970).

Whitney (1953) found that levels of blood flow obtained with the gauge on the human forearm under controlled conditions were of the same order as those obtained with the water plethysmograph under similar conditions; attempts to use both methods simultaneously on the same forearm gave anomalous results, however, possibly because mutual interference between the two instruments could not be avoided. Clarke and Hellon (1957) therefore made simultaneous observations of blood flow but with the gauge mounted on one forearm of a subject and a water plethysmograph mounted on the other; the results were recorded with the same measuring instrument and the authors found that over a wide range there was a small consistent difference in results in that, on the average, the water plethysmograph recorded flows 9.4% higher than those

recorded by the gauge method. In contrast, Paulev (1969) found in comparisons resembling those of Clarke and Hellon (1957) that the strain gauge gave higher resting flows. Horeman (1958) as well as Holling et al., (1961) claim to have obtained similar results when the strain gauge and water plethysmograph were employed under comparable circumstances on the forearm. It is of interest therefore in view of the latter that while the gauge was calibrated in situ on the limb under observation by Whitney (1953) and Clarke and Hellon (1957), Holling et al. (1961) carried out a "physical" calibration procedure. More recently Dahn and Hallbrook (1970) completed a detailed comparison of water and strain gauge plethysmography; these authors (Dahn and Hallbrook, 1970) found that practically equal values for blood flow could be obtained with each method provided that (1) blood flows were recorded with each instrument simultaneously on the same segment of the limb and (2) all plethysmograms were recorded with the same measuring instrument.

As noted in section (c) above, forearm blood flow in the present studies was determined by strain gauge plethysmography. With the exception of the technique for calibration of the gauge, flow determinations were carried out according to the method of Whitney (1953). Despite the documented evidence quoted above, it was deemed essential to compare the results obtained using the strain gauge employed in the present studies with those obtained by water plethysmography. In these experiments, a segment of the forearm bearing the gauge in the usual position was encased by a water-filled plethysmograph (water temperature 33°C), care being taken to ensure that the walls of the latter did not impinge on the gauge. Accordingly, blood flow in the same segment of the same forearm could be recorded simultaneously with the strain gauge and water plethysmograph on the same Type M8 Recorder in a manner analogous to that used in the calf by Dahn and Hallbrook (1970). The water plethysmograph enclosed a forearm volume which was

690 ml on the average compared to a mean of 1000 ml, being the volume under study during the GTTs when the gauge alone was applied. The water filling the plethysmograph was exposed to atmospheric pressure through a small funnel and variations in forearm volume within the apparatus were measured as changes in water pressure through a nylon catheter introduced into the inferior aspect of the plethysmograph and connected to a Statham 23D transducer. At least eight inflow readings were made under basal conditions in each of 10 normal volunteers and the mean flow calculated for each method in each subject. It was found that in the group as a whole, the flow (mean ± SEM) using the strain gauge was virtually identical to that obtained by means of water plethysmography (Table 7).

In view of the above considerations, it was felt that for the purposes of the present studies, the advantages to be gained by using the strain gauge outweighed the disadvantages.

Examples of typical plethysmographs are shown in Fig. 7.

(g) THE USE OF ARTERIO-VENOUS DIFFERENCES IN THE UNSTEADY STATE

Zierler (1961) has reviewed the effects of an unsteady state on the application of the Fick Principle for the calculation of the rate of tissue metabolism. Valid use of the Fick Principle depends not only on a steady blood flow but also on constant arterial concentrations and on constant tissue metabolism. Accordingly, in order to facilitate the interpretation of data, it has been urged (Zierler, 1961) that wherever possible, experiments should be designed so that arterial concentrations are changed stepwise from one steady state to another and that a time equal to the longest transit time through a system be allowed for the attainment of the new steady state. For the case of glucose, although the mean transit time through the forearm is 10 min,

TABLE 7

Comparison of blood flow (ml/100 ml forearm/min) obtained with mercury-in-rubber strain gauge and water plethysmograph

Subject	Strain gauge	Plethysmograph	Difference
1	4.65	4.13	+ 0.52
2	2.10	2.50	- 0.40
3	6.00	6.30	- 0.30
4	3.36	3.33	+ 0.03
5	3.35	2.62	+ 0.73
6	2.93	3.24	- 0.31
7	1.74	1.59	+ 0.15
8	1.55	2.31	- 0.76
9	2.59	2.09	+ 0.50
10	1.23	1.80	- 0.57
Mean (± SEM)	2.95 ± 0.47	2.99 ± 0.44	- .04 ± .16

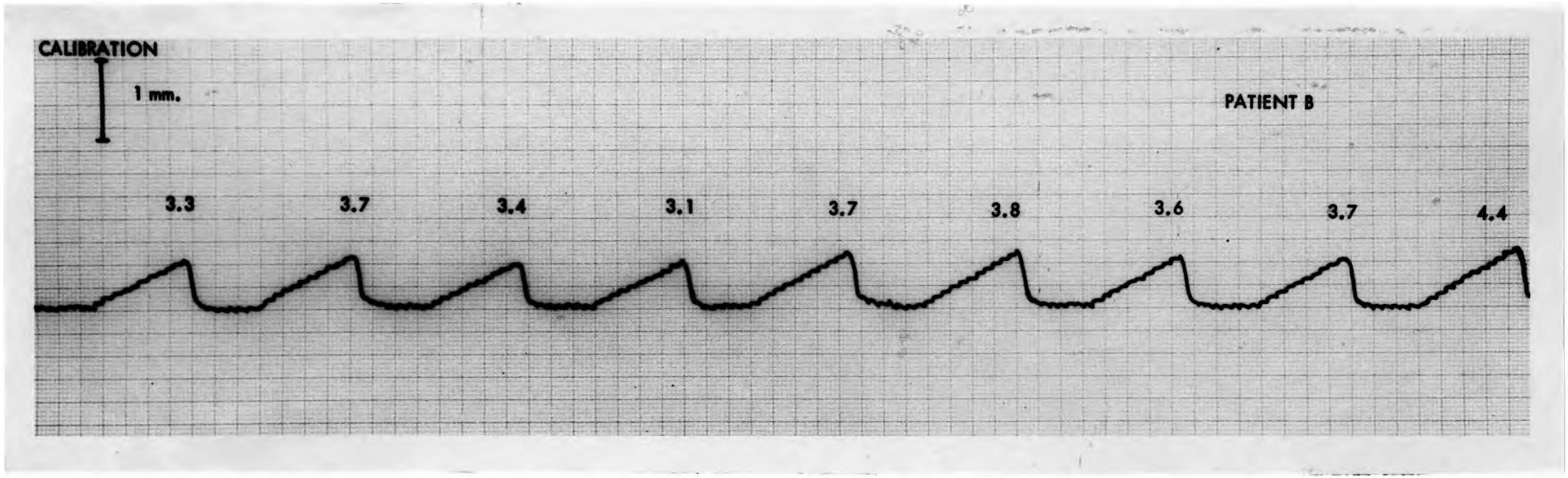

FIG. 7a Plethysmogram from a typical experiment showing the rate of blood flow (ml/100 ml forearm/min) indicated by each inflow recording

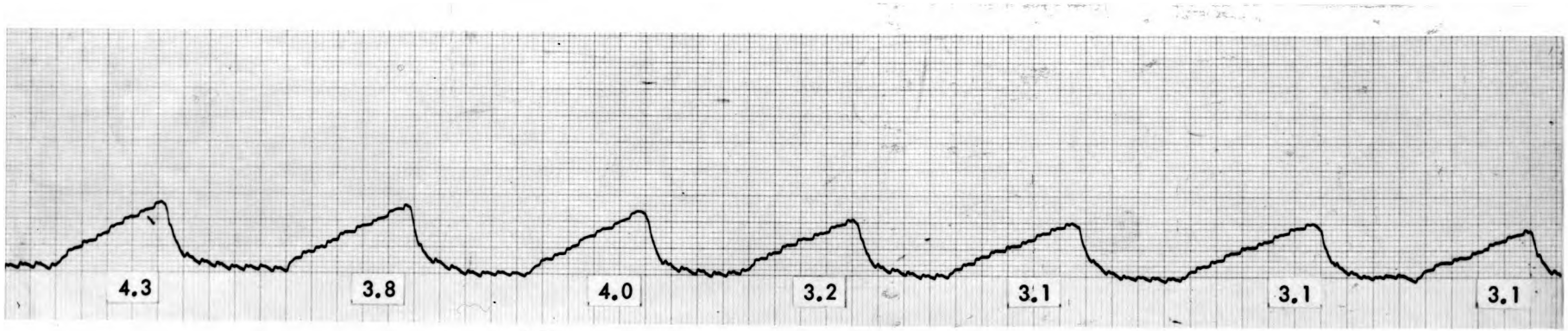

FIG. 7b Plethysmogram from a typical experiment showing the rate of blood flow (ml/100 ml forearm/min) indicated by each inflow recording

the longest transit time is about 30 min (Zierler, 1961).

It must be borne in mind, however, that Zierler's mathematical treatment of the problem, as the author himself points out, is not entirely appropriate to the highly complex biological situation (Harris et al., 1962). Transit times within the intravascular compartment are usually of the order of a few seconds, and this is supported by the findings of Butterfield and Holling (1959). During this short period after a change in blood flow or in arterial concentration, A-V differences will give an erroneous or even opposite measurement of the transfer of a metabolite into or out of the intravascular compartment. Beyond this time, the movement of the metabolite between the intra- and extravascular compartment is accurately followed by the A-V difference. The observations of Gowenlock et al. (1960) are an example of the relatively small error attached to an unsteady state within the intravascular compartment in the determination of the transfer of a solute into the tissues. Thus these authors found that following a single injection of K^{42} the estimate of tissue uptake in the forearm by means of A-V differences was very similar to the estimate derived from external counting.

While the exchange of metabolites between blood and tissue may be reasonably safely measured as the product of A-V differences and blood flow, significant errors may arise by equating the transfer of a metabolite to or from a tissue with its rate of metabolism within the tissue. This follows since the transit time of metabolites from the cells of a tissue to the capillaries and vice versa is considerably longer than the transit time of blood through the tissue. The movement of metabolites between the cells and the blood stream appears to be mainly determined by the diffusion constant of the substance, by the special relation between the cells and capillaries and by the ratio of tissue blood flow to tissue volume. The uncertainty which these

factors impose on the interpretation of A-V differences is greatest when only two such pairs of measurements are available but is considerably reduced by making a number of observations and interpreting the sequence of events as a whole.

In the present study, the principles enunciated by Zierler (1961) have been adhered to as far as the performance of GTTs will allow. Thus constancy of the rate of forearm blood flow throughout the experiments (see below) was achieved by (1) regulating the environmental air temperature to 21°-24°C during each experiment, (2) rejecting and subsequently repeating any experiment in which for any reason there were large fluctuations in blood flow and (3) circumventing the necessity for direct arterial puncture and accordingly, any of the possible unpleasant side-effects thereof. Furthermore, at no time were observations recorded at intervals greater than 15 min so that the sequence of events could be well defined.

(h) ANALYTICAL METHODS

Blood was collected in oxalate-fluoride tubes for glucose determinations; hormone assays were perfomed on serum (Henderson, 1970). Additional blood samples were collected in heparinised tubes and immediately cooled to 4°C. Plasma was separated from the latter in a refrigerated centrifuge (4°C) and aliquots extracted as soon as possible for free fatty acid (FFA) and triglyceride estimations - the remaining plasma was stored at -20°C and used subsequently for lactate, β-hydroxybutyrate and cholesterol determinations. Acetoacetate was determined on whole blood precipitated immediately on collection in ice-cold (4°C) perchloric acid (0.6M). It has been shown in separate experiments that during storage at -20°C lactate and β-hydroxybutyrate, but not acetoacetate, remain stable in plasma for at least five weeks (Table 8). It has been verified that

TABLE 8

Effect of storage at -20°C on plasma concentrations (nmol/l) of acetoacetate, β-hydroxybutyrate and lactate in subjects D.T. and R.M.

Duration of storage before estimation	Acetoacetate		β-hydroxybutyrate		Lactate	
Days	D.T.	R.M.	D.T.	R.M.	D.T.	R.M.
0	1.52	1.18	5.4	3.5	0.51	0.55
1	1.25	0.95	5.4	3.5	0.55	0.58
7	1.20	0.85	5.3	3.4	0.53	0.57
12	1.09	0.75	5.5	3.5	0.50	0.56
20	0.80	0.60	5.5	3.6	0.51	0.55
35	0.40	0.30	5.4	3.5	0.50	0.56

a similar pattern of change in lactate and -hydroxybutyrate concentrations across the forearm is found whether analyses are carried out on plasma stored at -20°C or on blood precipitated in ice-cold perchloric acid (0.6M) immediately on collection and that similar acetoacetate responses are observed whether estimations are performed on arterial or AV blood (Chapter 5, cf. Tables 18 and 19; Chapter 6, cf. Tables 24 and 26). FFA levels were determined on MV blood. All samples from the same experiment were always analysed together.

(1) Glucose

Glucose was estimated in triplicate on plasma (Zalme and Knowles, 1965; Tustison et al., 1966) by the ferricyanide method of Hoffman (1937) on a Technicon Auto-Analyser. In this procedure the determination of glucose is based on the photoelectric measurement of the diminution in color of a yellow potassium ferricyanide solution when it is reduced to colorless potassium ferrocyanide by glucose. The color is measured at 420 mμ using a flow cuvette with a 15 mm light path. This procedure proved reliable and accurate (Table 9).

(2) Insulin

Insulin was measured by a modification (Welborn and Fraser, 1965) of the double-antibody method of Morgan and Lazarow (1963). The principle of the assay is based on the observation that unlabelled insulin competitively inhibits the binding of I^{125}-labelled insulin to insulin antibodies. If identical amounts of I^{125}-insulin and an insulin antibody are added to a large number of tubes with different amounts of unlabelled insulin, an inverse but quantitative relationship will exist between the amount of unlabelled insulin added to the tube and the amount to I^{125}-insulin which is bound to the antibody. The latter can

TABLE 9

Variability of the automated ferricyanide method for the determination of glucose

Plasma glucose	Standard deviation	Coefficient of variation
mg/100 ml	mg/100 ml	%
40	± 1.8	4.5
84	± 2.5	3.0
156	± 3.5	2.2

be determined following precipitation of the insulin-antibody complex (which is soluble) with an anti-gamma globulin antibody; the radioactivity emitted by the precipitate can be measured. Addition of accurately measured amounts of a pure unlabelled insulin permits the construction of a standard curve by plotting the amount of radioactive insulin bound against the amount of added insulin.

The assay was performed by using borate buffer pH 8.0 containing per litre 8.25 g boric acid, 2.7 g sodium hydroxide, 0.1 g merthiolate and 0.2% albumen (Fraction V from bovine plasma - Armour Pharmaceutical Company Limited, Eastbourne, England). A human insulin standard (Medical Research Council) was used and standards and sera were assayed in a dilution of 1:10 (0.1 ml sample + 0.9 ml buffer). Serum samples were incubated with anti-insulin serum (Wellcome Reagents Ltd.) added in a dilution of 1:16,000; this incubation at 4°C continued for 16-24 hours before the addition of I^{125}-insulin. After further incubation at 4°C for 4 days, each tube received aliquots of guinea pig anti-insulin serum and rabbit anti-guinea pig gamma globulin; the latter was added

in amounts sufficient to achieve precipitation of all the guinea pig gamma globulin in the assay tubes. The tubes were then incubated ($4^{o}C$) for a further 16-24 hours before centrifugation ($4^{o}C$) at 2000 r.p.m. for 30 min. The supernatent fluid was decanted and discarded; the assay tubes containing the precipitate were retained for counting in an auto-gamma counter.

Examples of typical standard curves are shown in Figs 8(a) - 8(d); Fig. 8(c) shows, in addition, the influence of halving the concentrations of anti-insulin serum on the first half of the standard curve. Fig. 8(d) shows the influence on the standard curve of adding 0.1 ml saline to each assay tube on the first day of the assay. Table 10 shows the recovery of insulin from serum and Table 11 the results obtained after dilution of serum with buffer. Table 12 details the variability of the method within and between assays at different serum insulin concentrations. Table 13 compares the results obtained when serum withdrawn during a GTT was assayed in successive assays - the results emphasise the high degree of reproducibility of the method.

(3) Growth hormone

Growth hormone (GH) was assayed by a modification of the double antibody method of Hartog et al. (1964). Table 14 illustrates the variability of the method within and between assays (data supplied by Dr. A.D.Wright).

(4) Lactate

Lactate was determined according to modifications of well accepted semi-automated procedures (Antonis et al., 1966; Cramp, 1968). This method is based on the fluorimetric measurement of the increase in reduced nicotinamide-adenine dinucleotide (NADH) following the enzymatic

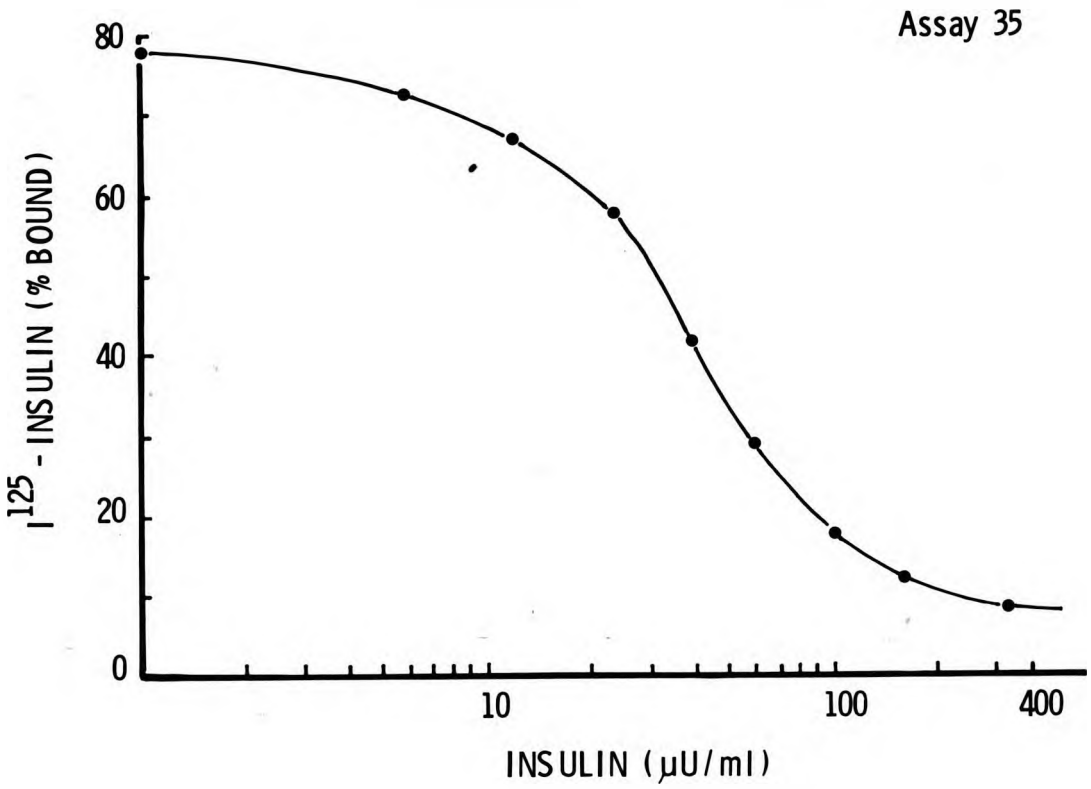

FIG. 8a Typical standard curve in the radioimmunoassay of insulin

conversion of lactate to pyruvate in the presence of lactic dehydrogenase (LDH).

$$\text{Lactate} + NAD^+ \rightleftharpoons \text{pyruvate} + NADH + H^+$$

This reaction is made to proceed from left to right in the presence of LDH and excess NAD^+ at pH 9.6 - 10.5 by trapping the pyruvate formed with semicarbazide or hydrazine hydrochloride as the ketone-trapping agent. This method is based on the fact that NADH has a characteristic native fluorescence, whereas the corresponding oxidised form has not.

(5) Acetoacetate and β-hydroxybutyrate

Acetoacetate and β-hydroxybutyrate were determined by modifications of a semi-automated procedure (Antonis et al., 1966).

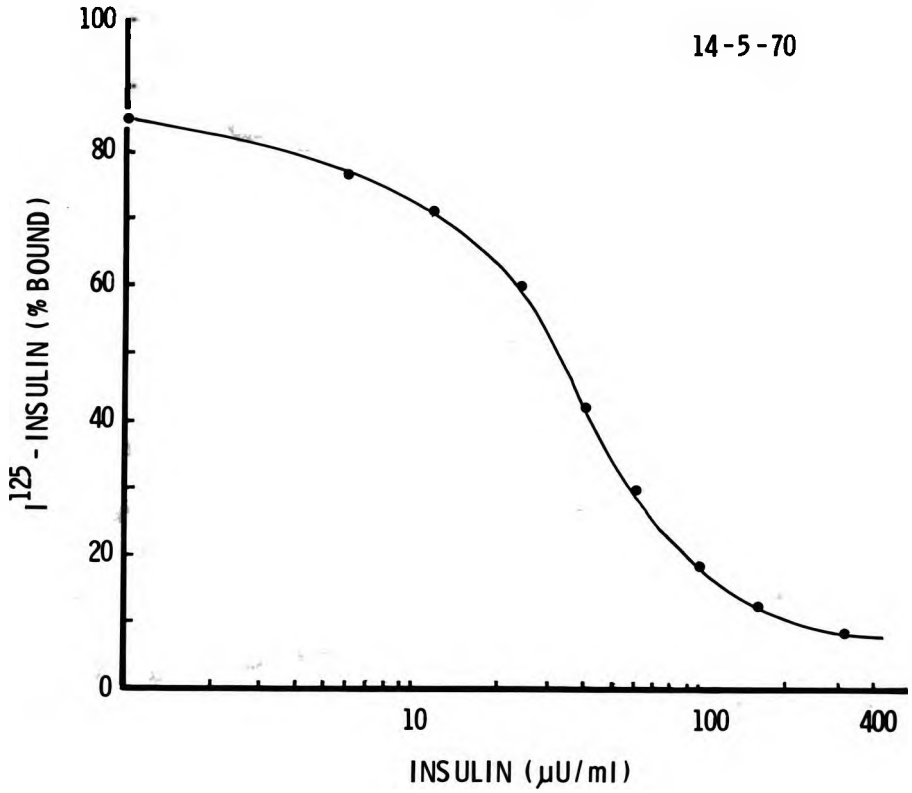

FIG. 8b Typical standard curve in the radioimmunoassay of insulin

This method depends on the reversible reaction catalysed by the enzyme -hydroxybutyrate dehydrogenase.

acetoacetate + NADH + H^+ $\rightleftharpoons$ β-hydroxybutyrate + NAD^+

and, as with the lactate method (see above), is based on the fluorimetric measurement of the change in NADH concentrations. The reaction proceeds rapidly to completion from left to right in the presence of excess NADH at pH 7, the reduction of the ketoacid being virtually quantitative. The reaction can be made to proceed to completion in the opposite direction in the presence of excess NAD at pH 8.5 if the ketoacid is removed by a ketone-trapping agent such as hydrazine or semicarbazide.

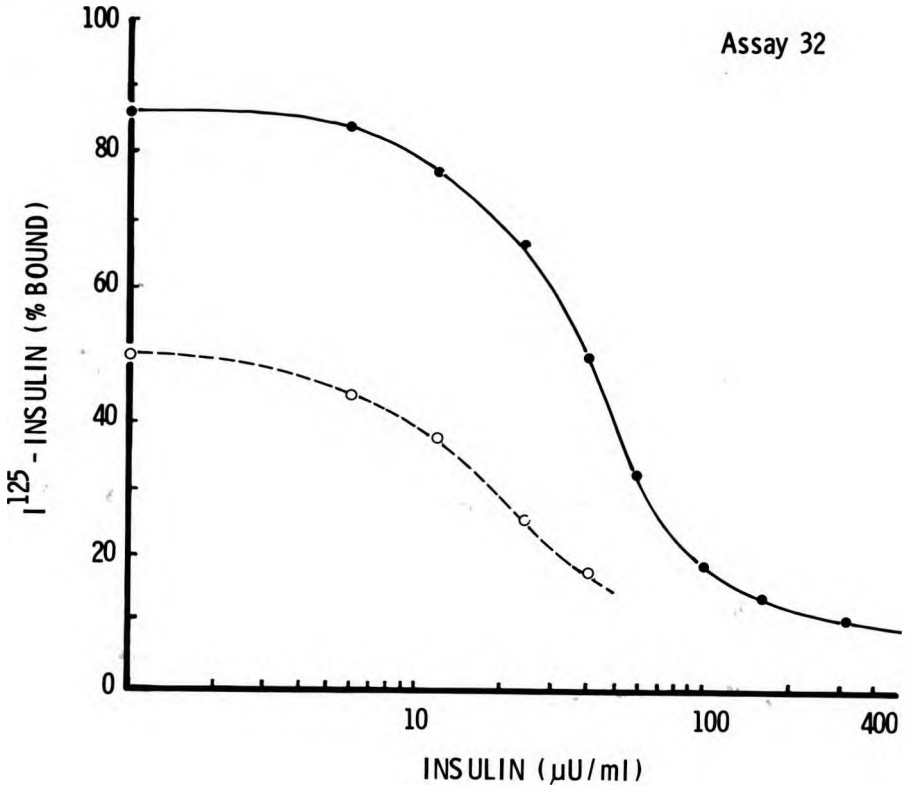

FIG. 8c Typical standard curves in the radioimmunoassay of insulin; anti-insulin serum was used in concentrations of 1:16,000 (●——●) and 1:32,000 (o- - -o)

(6) Free fatty acid

Free fatty acid (FFA) concentrations were determined by a semi-automated procedure (Antonis, 1965a, b) adapted from the method of Duncombe (1963). The procedure requires the preliminary preparation of a phospholipid-free plasma lipid extract (obtained with silicic acid and diisopropyl ether (Antonis, 1965b)) which has been reconstituted in chloroform; the chloroform solution of the FFA is then analysed automatically. The method is based on the solubility of copper soaps in organic solvents and the complexing of the copper with diethyldithiocarbamate. The specimen in chloroform is pumped together

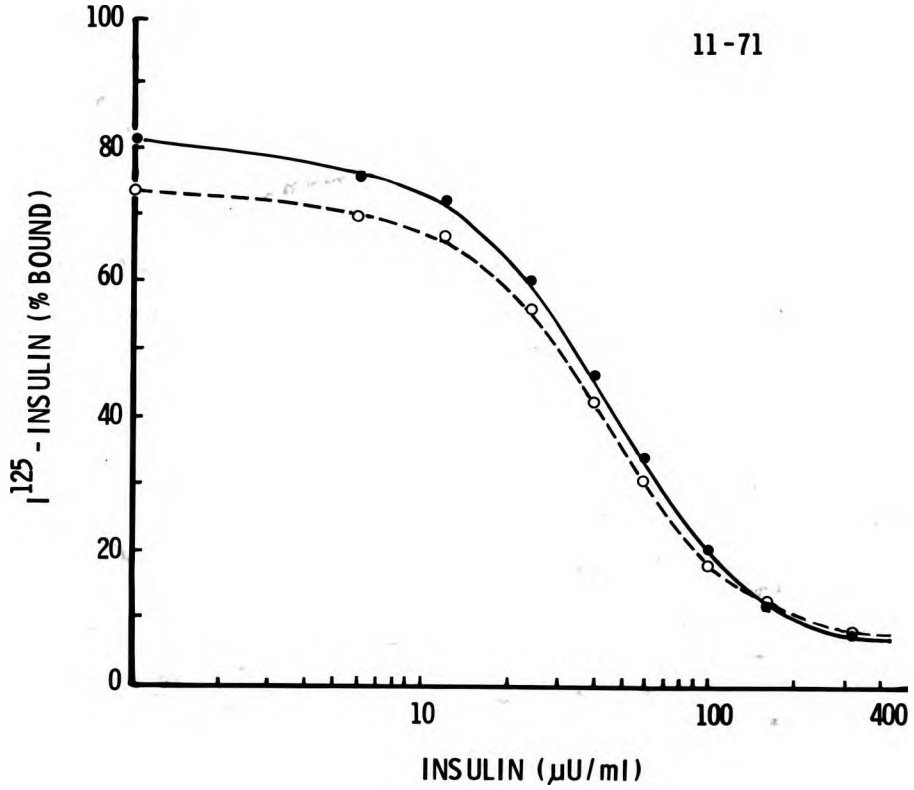

FIG. 8d Typical standard curve in the radioimmunoassay of insulin (●——●) and the influence on the curve of adding 0.1 ml saline to each assay tube on the first day of the assay (o- - - o)

with an aqueous copper nitrate - triethanolamine solution through a mixing coil filled with glass beads before being separated into two phases. The aqueous copper phase together with a portion of the chloroform phase passes to waste, and the remainder of the chloroform phase (containing the copper soaps) is redrawn through the pump and caused to react with a solution of sodium diethyldithiocarbamate in butanol before passing through the flow cell of a colormeter with subsequent recording of the extinction at 440 mμ.

TABLE 10

Recovery of added insulin from serum

Insulin added	Insulin recovered	Recovery
μU/ml	μU/ml	%
100	94	94
80	80	100
60	62	103
50	52	104
40	42	105
32	34	106
16	12	75
10	9.5	95

(7) Triglyceride

Triglyceride was estimated by a fluorimetric semi-automated procedure (Antonis, 1965a; Cramp and Robertson, 1968). The method is based on the Hantzsch condensation reaction between an amine, a β-diketone and an aldehyde. Triglyceride is saponified to glycerol and its oxidation with periodate produces formaldehyde which reacts with ammonia and acetylacetone to form 3,5-diacetyl-1,4-dihydrolutidine. The latter fluoresces with a maximum at 530 mμ when activated with light of 405 mμ. Phospholipid-free plasma extracts obtained with silicic acid and diisopropylether are reconstituted in isopropanol; the isopropanolic extract is added to an air-regimented stream of isopropanolic potassium hydroxide, and complete saponification to glycerol is achieved

TABLE 11

Serial dilution of serum with borate buffer

Sample	Dilution	Predicted concentration	Actual concentration
		μU/ml	μU/ml
A.	undiluted	-	140
	9:10	126	127
	8:10	112	112
	7:10	98	108
	6:10	84	92
	5:10	70	74
	4:10	56	62
	3:10	42	50
	2:10	28	32
B.	undiluted	-	38
	8:10	30	27
	6:10	23	21
	5:10	19	19
	4:10	15	17
	3:10	11	14

TABLE 12

Variability of the double-antibody method for the immunoassay of insulin

	Insulin	Standard deviation	Coefficient of variation
	μU/ml	μU/ml	%
Within assay	7	1.4	20.0
	12	5.8	48.0
	26	5.8	22.2
	31	3.4	11.0
	37	3.8	10.3
	57	1.7	3.0
	93	5.0	5.4
Between assays	52	6.2	11.9
	126	15.2	12.1

by passing through a 50°C heating bath. Following saponification both periodic acid and an acetylacetone reagent are added to the reaction mixture. The stream passes through a 50°C heating bath where glycerol is oxidised to formaldehyde and simultaneously the fluorescent condensation product is formed. The solution then passes through the flow cell of a fluorimeter where it is activated by ultra violet light at 405 mμ. The increase of fluorescence is measured at 505 mμ.

TABLE 13

Serum insulin response following 100 g oral glucose load: results (μU/ml) obtained in successive assays

Experiment		Minutes												
		0	15	30	45	60	75	90	105	120	135	150	165	180
I	First assay	3	11	25	29	45	49	43	38	25	27	22	15	11
	Second assay	11	14	24	32	45	44	37	39	28	27	23	17	12
II	First assay	5	-	40	-	57	-	56	-	24	-	12	-	-
	Second assay	4	-	34	-	56	-	52	-	24	-	14	-	-
III	First assay	4	13	16	18	28	26	25	25	22	24	22	22	15
	Second assay	13	16	22	22	30	30	27	28	24	22	22	20	12
IV	First assay	5	11	17	29	38	44	55	34	19	25	24	14	7
	Second assay	1	8	19	28	37	49	49	35	22	26	26	22	20
V	First assay	8	28	61	60	38	38	41	41	38	36	35	44	18
	Second assay	18	31	64	64	40	40	47	48	51	40	37	47	23

TABLE 14

Variability of the double-antibody method for the immunoassay of growth hormone (GH)

	GH	Standard deviation	Coefficient of variation
	ng/ml	ng/ml	%
Within assay	2.9	0.2	6.9
	7	0.2	2.9
	19.7	1.2	5.3
Between assays	5.5	1.2	21.8

(8) Cholesterol

Cholesterol was estimated by an automated technique (Antonis, 1965a; Block et al., 1965) based on the reaction between concentrated sulphuric acid and ferric chloride in acetic acid with steroids, such as cholesterol, which have a 5-ene, 3β-ol grouping. An anhydrous ferric chloride-sulphuric acid - acetic acid reagent is pre-heated before reacting with a phospholipid-free isopropanol extract of plasma or serum, and the extinction of the resulting solution is measured at 550 mμ.

(9) Statistical calculations

Statistical calculations were made using the paired or unpaired "t" test, depending on the specific analysis being carried out.

CHAPTER 5

THE RESPONSE TO GLUCOSE LOADING IN NORMAL SUBJECTS

This Chapter details the response to glucose loading in normal subjects following the standard dietary preparation.

(a) SUBJECTS

25 normal healthy volunteers were studied of whom 23 were male and two were female (Table 15). A family history of diabetes was not present in any of the group with the exception of two males each of whom had one diabetic parent; this factor alone, however, was considered insufficient to warrant their exclusion from the study (Pyke, 1968). Each subject continued normal daily activities and consumed a minimum of 220 g carbohydrate daily for at least five days prior to the performance of a GTT.

(b) RESULTS

(1) Response to glucose loading

Table 16 and Fig. 9 summarise the mean response during the GTTs. Peak concentrations of glucose and insulin were reached 30 min after glucose loading but peak rates of FGU were not achieved before the passage of yet a further 30 min. Although MV glucose levels had returned to the baseline by 135 min, AV glucose and serum insulin concentrations remained elevated even after 180 min. The data for basal FGU fall at the lower end of the range found by other workers (Andres et al., 1962; Rabinowitz and Zierler, 1962b; Butterfield et al., 1963; Butterfield and Whichelow, 1965) in normal subjects and this is explained by the use of AV rather than arterial blood. After glucose loading, FGU increased rapidly to reach a peak at 60 min; during the remainder of the test FGU decreased by only 50% so that a 10-fold elevation was still present at 180 min.

TABLE 15

Clinical data

Subject	Sex	Age	Height	Weight	Family history
		yr	cm	kg	
M.R.	M	20	168	62.1	nil
J.B.	M	25	179	65.3	nil
C.B.	F	23	162	50.1	nil
M.B.	M	20	175	63.9	nil
P.L.	M	20	183	75.5	nil
J.H.	M	20	171	68.7	father diabetic
J.L.	F	23	160	54.9	nil
G.M.	M	21	175	76.4	nil
R.M.	M	20	179	76.8	nil
D.S.	M	20	180	73.8	nil
D.T.	M	21	183	74.0	nil
A.Y.	M	23	173	67.0	nil
C.H.	M	23	176	72.0	nil
D.M.	M	22	178	66.3	nil
C.K.	M	22	173	62.4	nil
M.B.	M	22	186	72.9	nil
G.T.	M	20	163	67.5	nil
B.S.	M	21	191	76.9	nil
A.P.	M	42	179	70.0	nil
J.R.	M	32	163	53.5	nil
R.H.	M	21	191	91.1	nil
J.M.	M	20	177	76.5	nil
J.S.	M	22	168	68.5	nil
T.P.	M	47	176	73.9	father diabetic
C.D.	M	25	188	68.8	nil
Mean		23.8 $\pm$ 1.4*	176 $\pm$ 2	68.9 $\pm$ 1.7	

*Standard error of mean

TABLE 16

Mean response following 100 g oral glucose load in 25 normal subjects

		Minutes					
		0	15	30	45	60	75
Glucose mg/100 ml	AV*	86 ± 1**	124 ± 3	162 ± 4	161 ± 5	151 ± 6	139 ± 6
	MV§	84 ± 1	102 ± 2	127 ± 3	121 ± 5	111 ± 6	103 ± 5
Glucose Uptake mg/100 ml forearm/min		.029 ± .005	.289 ± .042	.449 ± .042	.549 ± .036	.607 ± .042	.572 ± .051
Insulin µU/ml		11 ± 1	39 ± 4	78 ± 7	69 ± 5	71 ± 8	72 ± 11
FFA µmol/l		444 ± 30	-	345 ± 25	-	200 ± 11	-
Growth hormone ‡ ng/ml		6.6 ± 2.1	-	2.7 ± 0.5	-	2.5 ± 1.0	-
Blood flow ml/100 ml forearm/min		2.4 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.4 ± 0.2	2.7 ± 0.2	2.7 ± 0.2

* Arterialised venous

** Standard error of mean

§ Mixed venous

‡ n = 21

TABLE 16 Cont.

Mean response following 100 g oral glucose load in 25 normal subjects

		Minutes						
		90	105	120	135	150	165	180
Glucose mg/100 ml	AV*	130 ± 4	121 ± 3	116 ± 3	110 ± 3	110 ± 4	104 ± 2	102 ± 3
	MV§	98 ± 4	92 ± 4	91 ± 3	84 ± 3	86 ± 3	86 ± 3	86 ± 3
Glucose uptake mg/100 ml forearm/min		.533 ± .046	.484 ± .039	.462 ± .038	.420 ± .038	.424 ± .035	.349 ± .029	.294 ± .029
Insulin µU/ml		64 ± 7	58 ± 7	54 ± 7	48 ± 8	44 ± 4	39 ± 4	35 ± 5
FFA µmol/l		72 ± 11	-	158 ± 8	-	154 ± 7	-	166 ± 11
Growth hormone† ng/ml		2.5 ± 1.0	-	2.1 ± 0.5	-	3.2 ± 1.3	-	5.1 ± 1.6
Blood flow ml/100 ml forearm/min		2.9 ± 0.2	2.9 ± 0.2	3.2 ± 0.2	2.9 ± 0.2	3.1 ± 0.2	3.4 ± 0.2	3.4 ± 0.3

* Arterialised venous

** Standard error of mean

§ Mixed venous

† n = 21

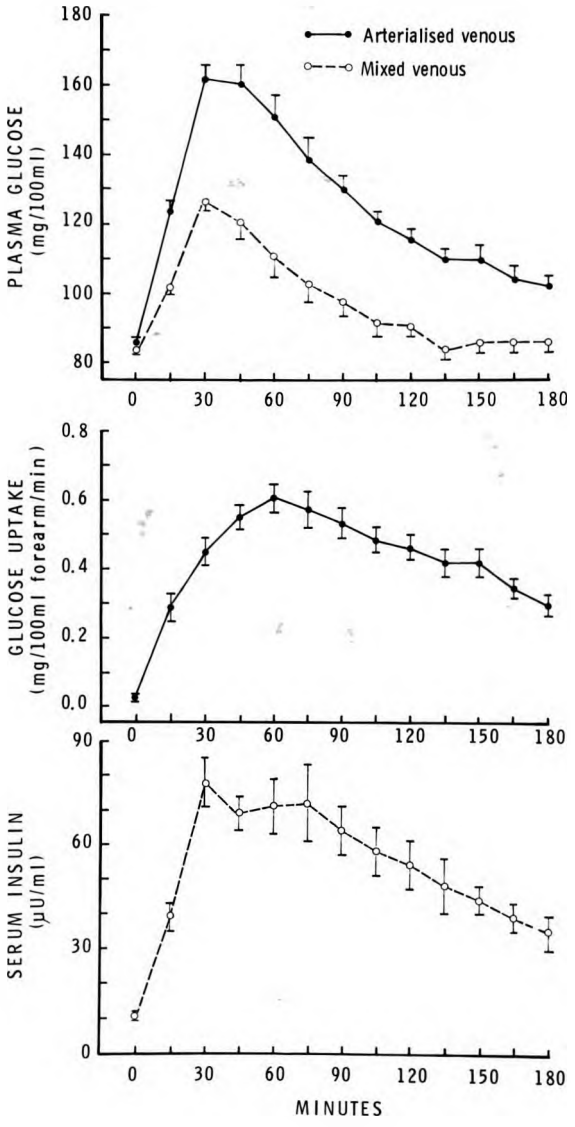

FIG. 9 Mean (±SEM) response following 100 g oral glucose load in 25 normal subjects

When the individual responses during the GTTs were expressed as incremental areas (i.e. the area enclosed by the appropriate response curve above the basal concentration), correlations were not found in any combination between the changes in AV glucose levels, insulin concentrations and FGU. Fig. 10 (see Appendix, Table 3) shows, however, that a highly significant negative correlation was found between peripheral tissue responsiveness to insulin ($\Delta FGU/\Delta I$) and the β-cell response to glucose ($\Delta I/\Delta G$). Only slight changes were detected in mean forearm blood flow after glucose administration - after an initial fall there was a gradual rise but throughout the GTT flow remained fairly constant between 2.1 - 3.4 ml/100 ml forearm/min, the mean ($\pm$SEM) being 2.8 $\pm$ 0.2. The mean ($\pm$SEM) coefficient of variation for blood flow within an individual during the GTT was 20.7 $\pm$ 1.2%. Basal FFA concentrations were reduced by 65% during the first hour and by a further 10.4% during the subsequent 90 min; no rebound had become apparent when the test was terminated. Serum growth hormone levels remained low throughout, falling initially but rising again during the final 60 min of testing.

In 13 subjects, insulin and growth hormone concentrations were measured in both AV and MV blood (Fig.11; Table 17); significant AV-MV differences were shown across the forearm for insulin between 30 and 150 min but not at any stage for growth hormone. When expressed as a percentage of the associated AV insulin concentrations, the AV-MV insulin differences amounted to 27%, 19%, 19%, 15% and 11% at 30, 60, 90, 120 and 150 min respectively. The AV insulin levels were positively correlated with forearm insulin uptake at 30 min ($r = .85$; $y = .56x - 9.03$; $p < .01$) and 90 min ($r = .58$; $y = .26x + 1.93$; $p < .05$) and with AV-MV insulin differences at 30 min ($r = .70$; $y = .21x + 6.69$; $p < .01$), 60 min ($r = .79$; $y = .37x - 16.21$; $p < .01$) and 90 min ($r + .75$; $y = .28x - 6.67$; $p < .01$); the raw data are detailed below (see Appendix, Table 4). Fig. 12

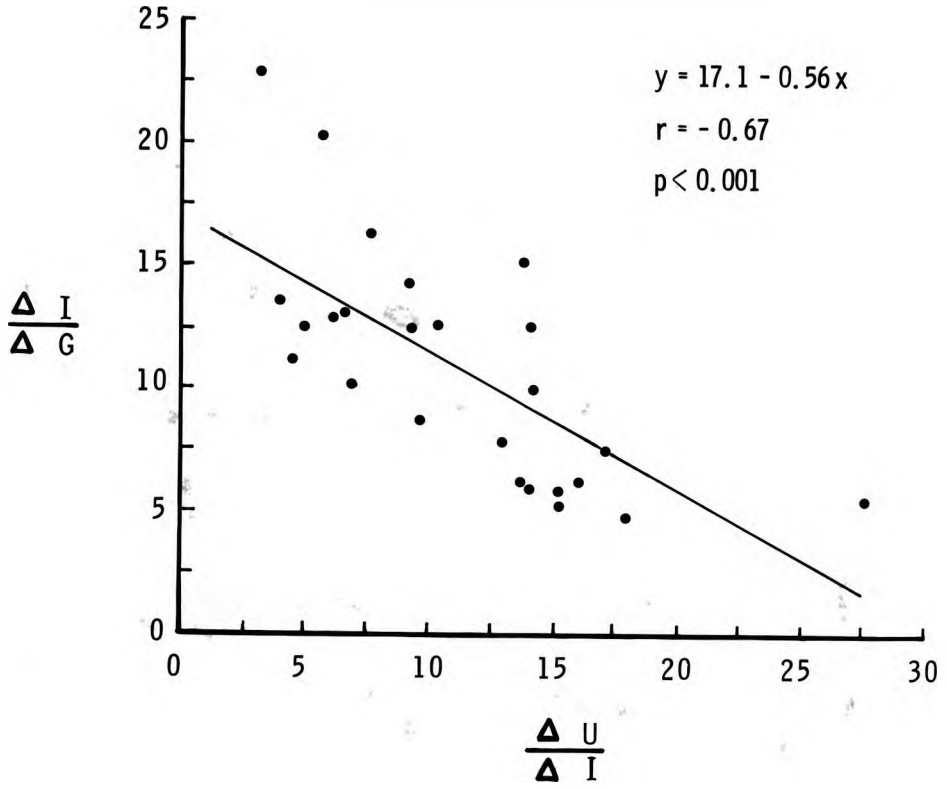

FIG. 10 Correlation between the insulin response to glucose ($\Delta I/\Delta G$) and the peripheral tissue response to insulin ($\Delta U/\Delta I$) where ΔI, ΔG and ΔU are the incremental areas under the curves for serum insulin, arterialised venous and forearm glucose uptake shown in Fig. 9

(Table 17) shows that in these 13 subjects, there was a marked rise in both glucose and insulin uptake by the forearm at 30 min; despite an equally rapid fall-off in insulin uptake by some 50% during the following half-hour, the associated high levels of FGU were maintained for a further 90 min before gradually declining. Furthermore, this fall-off in forearm insulin uptake took place despite relatively sustained AV insulin concentrations. A significant correlation was not found between FGU and forearm insulin uptake when expressed as

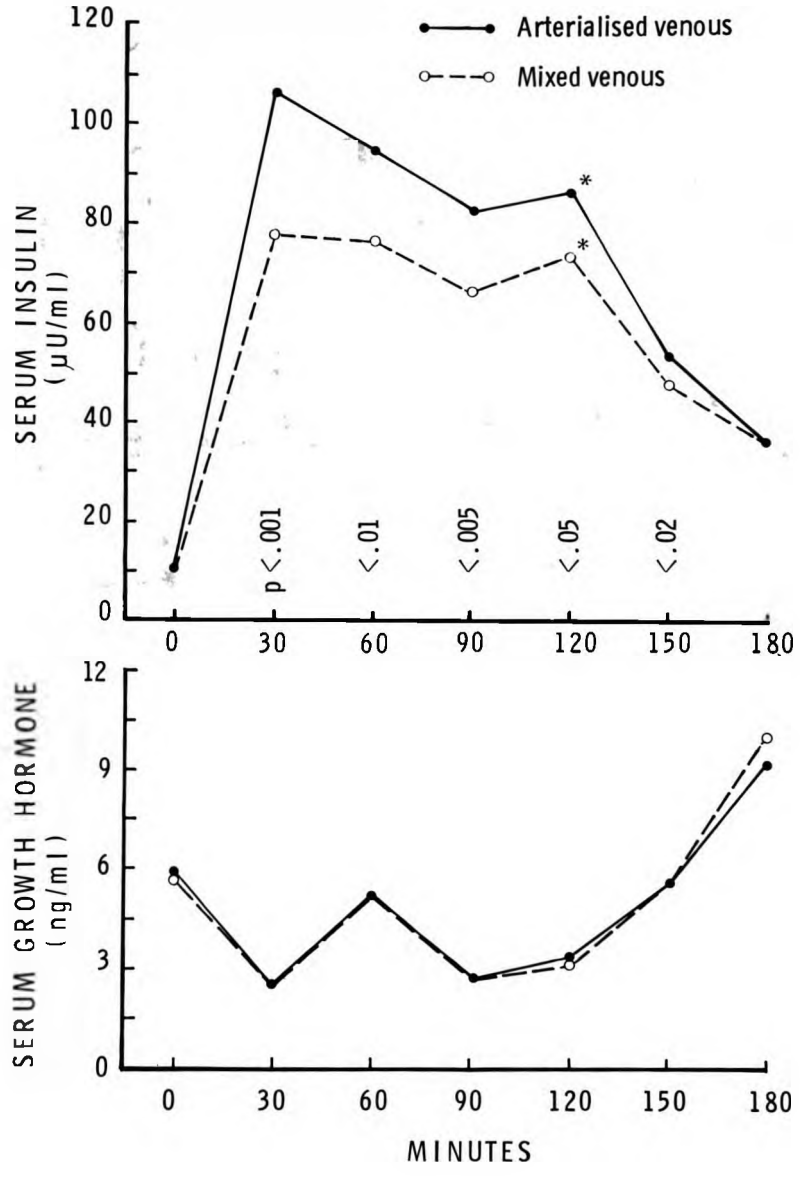

FIG. 11 Mean changes in insulin and growth hormone concentrations across the forearm following 100 g oral glucose load in 13 normal subjects

*n = 12 for this pair of observations

TABLE 17

Mean response following 100 g oral glucose load in 13 normal subjects

		Minutes						
		0	30	60	90	120	150	180
Insulin	AV*	11 ± 1**	107 ± 17	95 ± 11	83 ± 11	87 ± 19 ǂ	54 ± 6	36 ± 5
μU/ml	MV§	10 ± 1	78 ± 14	77 ± 7	67 ± 9	74 ± 14 ǂ	48 ± 6	36 ± 6
	p ǂ	N.S.	<.001	<.01	<.005	<.05	<.02	N.S.
Insulin uptake								
μU/100 ml forearm/min		1.6 ± 0.7	50.6 ± 11.5	26.2 ± 5.9	23.4 ± 5.2	24.7 ± 10.1	12.6 ± 4.3	4.2 ± 1.2
Glucose uptake								
mg/100 ml forearm/min		.038 ± .009	.514 ± .057	.560 ± .070	.489 ± .050	.485 ± .061	.407 ± .069	.316 ± .056
Growth hormone	AV	5.9 ± 3.0	2.6 ± 0.8	5.3 ± 2.8	2.7 ± 0.7	3.3 ± 0.9	5.6 ± 2.0	9.2 ± 2.8
ng/ml	MV	5.6 ± 2.7	2.6 ± 0.8	5.3 ± 2.8	2.7 ± 0.8	3.2 ± 0.9	5.6 ± 2.1	10.1 ± 3.3
	p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Arterialised venous
** Standard error of mean
§ Mixed venous
ǂ Significance of difference between means
ǂ n = 12

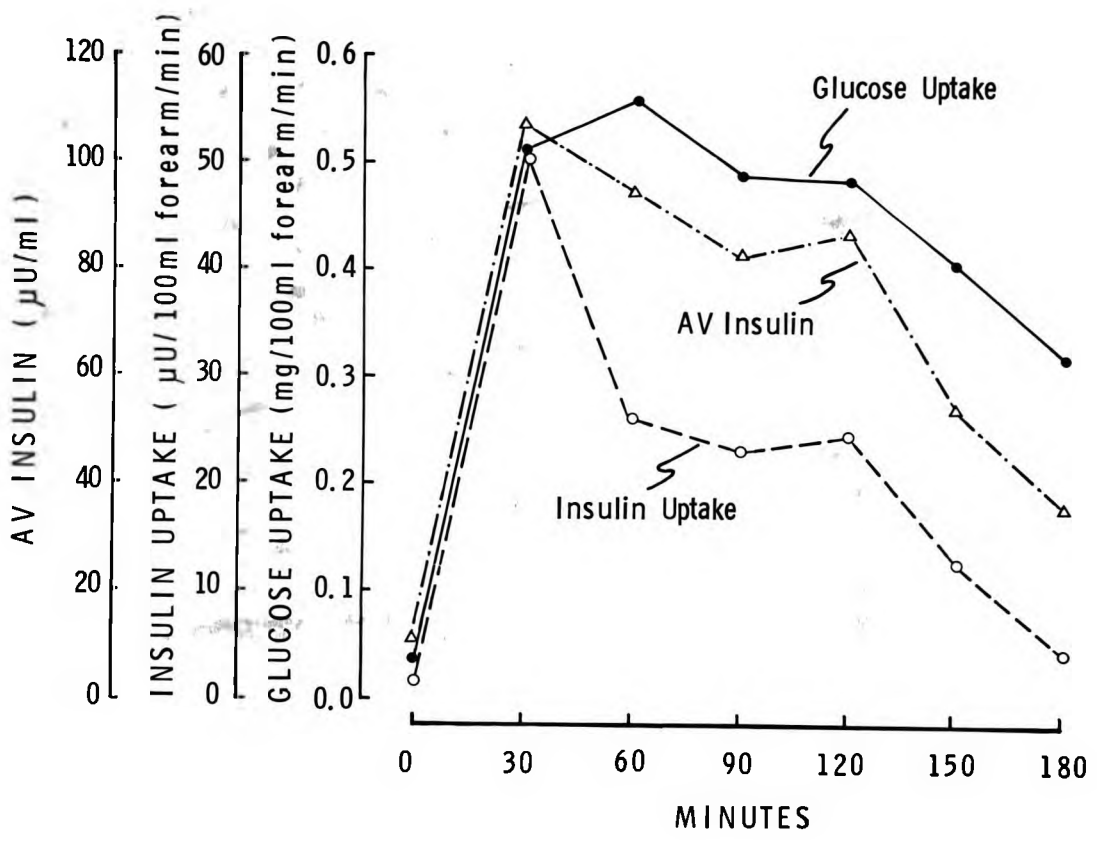

FIG. 12 Relationship between mean arterialised venous insulin concentrations, forearm insulin uptake and forearm glucose uptake in 13 normal subjects

the incremental areas 0 - 180 min.

The plasma lactate response was studied in 11 subjects (Fig. 13; Table 18); lactate concentrations rose rapidly to a peak 60 min after glucose loading and fell thereafter. Basally, lactate was neither taken up nor released by the forearm; during the rise in AV concentrations, lactate was taken up by the forearm tissues and released in association with falling AV levels during the final 30 min. The failure to detect lactate release under basal conditions is in contradistinction to the findings of other workers (Rabinowitz and Zierler, 1962b) and must be

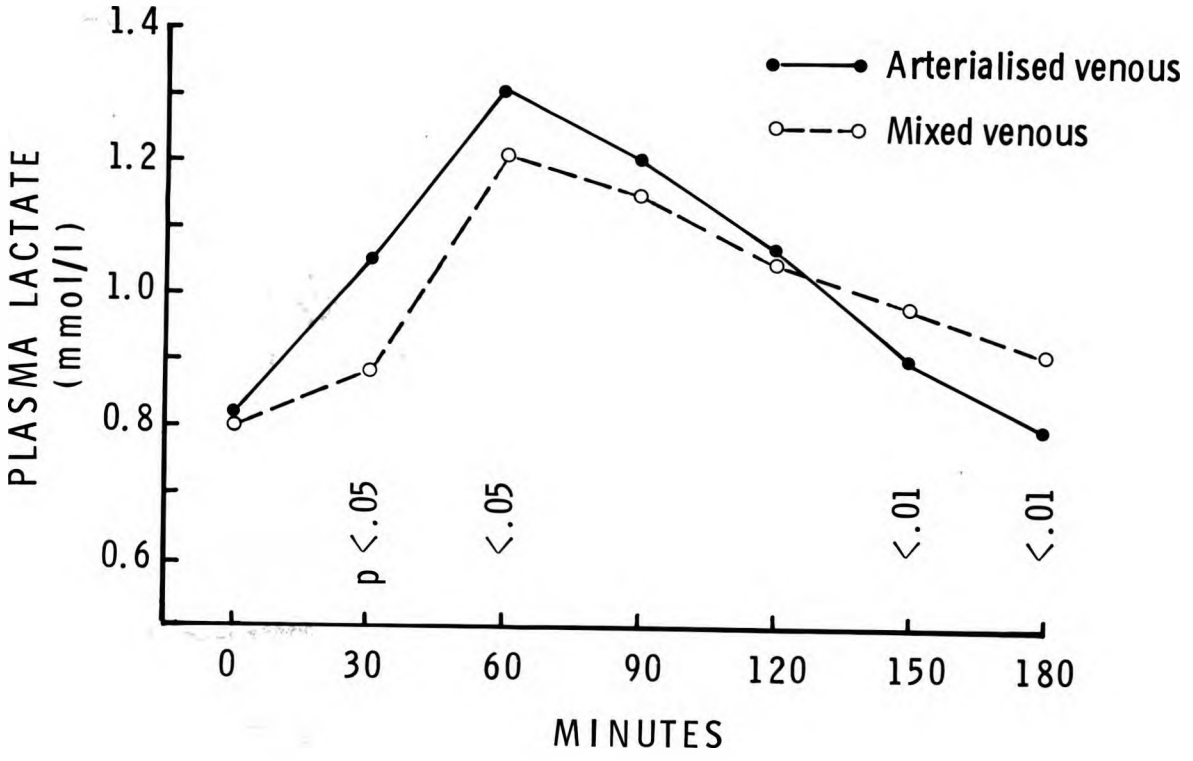

FIG. 13 Mean changes in lactate concentrations following 100 g oral glucose load in 11 normal subjects

attributed to the use of AV rather than arterial and MV rather than deep venous blood. This only serves to emphasise, however, that the findings of positive AV-MV lactate differences at 30 and 60 min provide, if anything, an underestimate of forearm lactate removal. Similar patterns of response across the forearm are obtained whether estimations are performed on plasma or whole blood precipitated immediately on collection in ice-cold 0.6M perchloric acid (cf. Tables 18 and 19).

TABLE 18

Mean lactate concentrations (mmol/l) in arterialised venous (AV) and mixed venous (MV) plasma following 100 g oral glucose load in 11 normal subjects

	Minutes						
	0	30	60	90	120	150	180
AV	0.82 ± .07*	1.05 ± .11	1.31 ± .11	1.21 ± .10	1.07 ± .08	0.90 ± .07	0.80 ± .06
MV	0.81 ± .07	0.89 ± .06	1.21 ± .09	1.15 ± .07	1.05 ± .09	0.98 ± .07	0.91 ± .07
p**	N.S.	$<.05$	$<.05$	N.S.	N.S.	$<.01$	$<.01$

* Standard error of mean

** Significance of difference between means

TABLE 19

Lactate concentrations (mmol/l) in arterialised venous (AV) and mixed venous (MV) blood following 100 g oral glucose load

Subject		Minutes						
		0	30	60	90	120	150	180
J.R.	AV	0.54	0.85	0.93	0.88	0.80	0.55	0.56
	MV	0.54	0.65	0.86	0.87	0.85	0.69	0.64
T.P.	AV	0.92	1.17	1.45	1.35	-	-	0.73
	MV	0.88	0.91	1.16	1.17	-	-	0.92

(2) Fate of the ingested glucose load

It is generally accepted that ingested glucose is taken up by the body in two major sites - the liver on the one hand and peripheral tissues on the other (Cahill et al., 1959; Perley and Kipnis, 1967).

From the area under the glucose uptake curve (Fig. 9) it may be calculated that the mean (+SEM) increment in FGU during the 180 min after glucose loading was 74 ± 4.7 mg/100 ml forearm (range 29.4 - 120.0 mg/100 ml forearm). The extent to which the peripheral tissues in the body as a whole contribute to the disposition of the glucose load can be approximated from this data. Firstly, assuming that total forearm blood flow is distributed between the deep and superficial compartments of the forearm in the ratio of 4:1 (Cooper et al., 1955), it may be deduced from the observations of others (Rabinowitz and Zierler, 1962b; Whichelow et al., 1967; Pozefsky et al., 1969), that muscle accounts for 89% of total FGU and adipose tissue the remaining 11%. This estimate seems eminently reasonable in terms of the present observations since the large AV-MV glucose differences (the largest was 78 mg/100 ml recorded in one subject 45 min after glucose loading) suggest that the bulk of the glucose taken up by the forearm, was indeed accounted for by muscle. On this basis and on the assumptions that (1) in non-obese subjects muscle and adipose tissue occupy respectively 60% and 8% of forearm volume (Abramson and Ferris, 1940; Cooper et al., 1955), and (2) the body of a normal 70 kg man contains 30 kg muscle (Andres et al., 1956; Butterfield and Whichelow, 1965) and 10 kg adipose tissue (Dole, 1965; Durnin, 1969), it may be calculated that during the GTTs (Table 16) the mean increment in total peripheral glucose utilisation amounted to some 43.2 g glucose; of this 43.2 g 76% (33 g) was taken up by muscle and 24% (10.2 g) by adipose tissue. Furthermore, it would appear that when considered hour by hour, the

increase in total peripheral glucose utilisation, though fairly evenly distributed in time, was maximal during the second half of the GTT, the relevant figures being 13.0 g in the first hour, 17.5 g in the second hour and 12.7 g in the third hour.

Using this data, a "balance sheet" may be drawn up which outlines the dispoition of the entire glucose load during the 180 min following ingestion (Table 20). It is inevitable, by virtue of the assumptions made, that the calculations (Table 20, Fig. 14 below) can be no more than gross approximations. These calculations nevertheless serve the important function of allowing the description of a pattern of events whereby the relative roles of the body tissues in glucose disposal may be assessed. The amount by which the glucose pool remained expanded at 180 min viz. 3.4 g, was estimated by multiplying the increment in AV glucose concentrations persisting at this time by the glucose space (Combes et al., 1961); the latter was assumed to be 30% of body weight (Reichard et al., 1961; Cahill et al., 1966; Forbath and Hetenyi, 1966). The studies of Perley and Kipnis (1967) and those reviewed by Holdsworth (1969) suggest that the absorption of 100 g glucose is completed long before the passage of 180 min and would not be rate-limiting in normal subjects. There is nevertheless the suggestion from animal experiments (Kiyasu et al., 1956; Shoemaker et al., 1963) though unconfirmed in man (Holdsworth and Dawson, 1965), that a minor fraction of the ingested glucose may be utilised in its passage through the gut wall; for the purposes of the present discussion, however, the small loss of glucose which might occur in this way has been ignored.

I have assumed, therefore, that the balance of the glucose load, i.e. 53.4 g, was accounted for by the hepatic response which consists of both an increase in hepatic glucose utilisation and a decrease in hepatic glucose production (Mebane et al., 1961; Bishop et al., 1965).

TABLE 20

Suggested "balance sheet" for the disposition of 100 g glucose following oral administration in normal subjects

		g
Unabsorbed after 180 min		0.0
Utilised in passage through gut		0.0
Total peripheral glucose uptake		43.2
(i) uptake by skeletal muscle	33.0	
(ii) uptake by adipose tissue	10.2	
Total hepatic glucose conservation		53.4
(i) suppression of hepatic glucose output	12.6	
(ii) hepatic glucose uptake	40.8	
Remaining in glucose space at 180 min		3.4
Loss in urine		0.0
		100.0

relevant to the 100 g GTT in man remain unavailable but allowing for a basal hepatic glucose output of 120 mg/kg/hour (Reichard et al., 1961; Forbath and Hetenvi, 1966; Paul and Bortz, 1969; Kreisberg et al., 1970) and a mean suppression of the latter during the GTT by at least 50% (Madison et al., 1960; Combes et al., 1961; Madison et al., 1963; Steele et al., 1968), it may be estimated that some 12.6 g would be accounted for by decreased hepatic glucose uptake. Since in six subjects only was there a short lived elevation in AV glucose levels above 180 mg/100 ml, it is considered that the mean urinary loss of glucose during the GTT in the group as a whole was negligible.

On this basis, Table 20 shows that the mean relative contributions of the liver and peripheral tissues to glucose homeostasis were 53.4% and 43.2% respectively. These proportions varied considerably among individuals, however, the estimates for total peripheral glucose utilisation ranging from 16.4 to 67.4 g glucose with corresponding values for total hepatic glucose conservation being 83.6 g and 32.6 g respectively.

Since the plasma glucose concentrations after glucose ingestion represent the balance between glucose entry into the general circulation and glucose uptake by peripheral tissues, and since the latter is known, the increment in the rate of glucose entry into the systemic circulation during the GTTs may be calculated. Incremental glucose entry was determined as the sum of peripheral glucose uptake and the change in the glucose pool per unit time; the latter, as noted above, is the product of the change in AV glucose concentrations and the glucose space. Fig. 14 (see Appendix, Table 5) shows that glucose entry was markedly increased during the periods 0-15 and 15-30 min but thereafter was strikingly reduced throughout the remainder of the test. Overall, some 45 g extra glucose entered the peripheral circulation during the GTT and together with the 12.6 g replaced

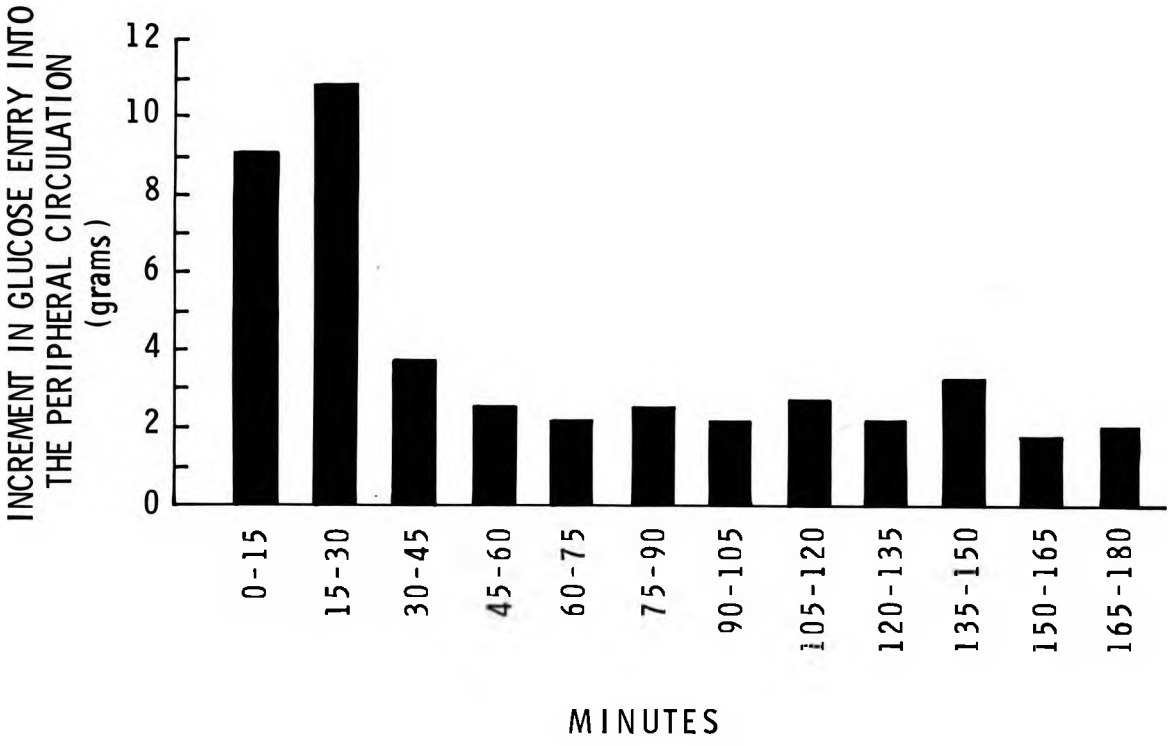

FIG. 14 Mean increment in glucose entry into the peripheral circulation following 100 g oral glucose load in 25 normal subjects

following the suppression of endogenous glucose production, it may be concluded that of the 100 g glucose ingested, some 57.6 g reached the periphery within 180 min.

(c) DISCUSSION

The present findings suggest that within 180 min of administration, *some 43.2% of a 100 g oral glucose load is taken up by peripheral tissues, 53.4% is accounted for by hepatic glucose conservation (Madison 1969), while the residual 3.4% remains within the glucose space (Table 20). Several previous attempts have been made to quantitate tissue glucose disposal during the oral GTT but estimates vary widely. This*

is not surprising since a variety of techniques have been employed and in each instance, as in the present study, the relevant calculations have involved making several assumptions. The limitations of isotope dilution techniques (Searle and Chaikoff, 1952; Reichard et al., 1958; Berson et al., 1959; Steele et al., 1968) in this regard are particularly serious since they measure only new glucose production by the liver, they cannot distinguish hepatic from peripheral glucose utilisation and as a result of equilibration of the isotope after absorption they will overestimate the amount of ingested glucose actually reaching the peripheral circulation. Using other methods, Scow and Cornfield (1954) reported that in rats, approximately 60-65% of an oral glucose load was taken up by the liver, the remainder entering the systemic circulation. Similar conclusions were reached by Perley and Kipnis (1967) in human subjects; by infusing 31 g glucose, these workers were able to reproduce the 100 g oral glucose tolerance curve and estimated that about two-thirds of the oral load must be handled by the liver and only one-third by peripheral tissues. The latter data is wholly consistent with the present study and the recent work of Felig and Wahren (1971) in man, which emphasises the liver as a primary target organ for achieving glucose homeostasis. Arnould et al., (1965) have shown in dogs that the liver stores 20-70% of the glucose reaching portal blood and this is in excellent agreement with the range of 32.6-83.6% calculated in the present investigation. Madison and his co-workers (Madison et al., 1963; Grivas and Madison, 1964; Madison, 1969) have concluded that net hepatic glucose conservation in dogs accounts for 28-30% of large glucose loads, but pointed out that since these animals had porto-caval shunts, they had probably underestimated the physiological role of the liver in the intact organism following a carbohydrate meal. More recently, Jeffcoate and Moody (1969) estimated in rats that liver glycogen formation accounted for 27%

of the absorbed glucose while Shoemaker et al. (1963) concluded in dogs that changes in hepatic glucose balance were responsible for the disposal of all the extra glucose. In contrast Butterfield and Whichelow (1965) and Whichelow and Butterfield (1971) ascribed only a minor role to the liver in the assimilation of a 50 g oral glucose load in lean nondiabetic subjects, and suggested that no less than 80-92% of the load was taken up in peripheral muscle.

The latter are the only available studies directly comparable with the current data. Butterfield and Whichelow (1965) calculated peripheral glucose utilisation from arterio-deep venous (A-DV) glucose differences and determinations of total forearm blood flow. However, because of the prevalent fluctuation in their studies of total flow over an unacceptably wide range [i.e. ranging up to 8.4 ml/100 ml forearm/min in one paper (Butterfield and Whichelow, 1965) and 12.4 with a mean of 5.6 in another (Whichelow and Butterfield, 1971)] and because, despite this, the data were not corrected to express muscle flow only, it is likely that these authors have grossly overestimated the magnitude of the skeletal muscle response. This follows from the findings of Cooper et al. (1955) who have demonstrated that when total forearm blood flow (expressed as ml/100 ml forearm/min) is 3.0 or less, some 80% of the blood perfuses the deep compartment of the forearm and with total flows between 3.0 - 6.0, roughly 73%, but when total flow rises to 6.0 or more, this fraction falls to 50% or less. Consequently, unless total forearm flow remains consistently basal throughout an experiment, its use, uncorrected, in conjunction with A-DV differences may result in the overestimation of muscle glucose uptake by 100% or more. Despite the contrast in technique employed, however, there is remarkably good agreement between the present findings and those of Perley and Kipnis (1967) and Scow and Cornfield (1954) and it would seem reasonable to conclude, therefore, that quantitatively the

peripheral and hepatic contributions to glucose disposal during the 100 g oral GTT commonly fall within the range indicated by these three studies.

The components of the hepatic response to glucose loading have not been studied comprehensively in man. Animal experiments indicate that there is both a reduction in glucose output and an increase in glucose uptake and that of the glucose taken up, some 90% is converted to glycogen (Mebane et al., 1961; Madison et al., 1963; Grivas and Madison, 1964; Bishop et al., 1965; Steele et al., 1968; Madison, 1969). Our lactate data (Fig. 13) suggest however that in the normal liver, this may be an overestimate i.e. that more than 10% of the glucose taken up may be metabolised by glycolysis and that indeed the plasma lactate response may prove a useful, albeit rough, index of hepatic glucose utilisation during the oral GTT. Thus, although it is well accepted that enhanced tissue glycolysis is the basis for the elevations in blood lactate and pyruvate concentrations found in normal subjects after glucose loading, the tissues specifically involved in this glycolysis have not been clearly defined (Sussman, 1966; Doar et al., 1968, 1970). Since glycolysis is a major metabolic pathway in muscle (Ashmore et al., 1960; Randle et al., 1966), and since it is generally accepted that in health significant lactate production is a function neither of liver (Cahill et al., 1959) nor adipose tissue (Renold et al., 1965), Sussman's proposal (1966), that muscle might be the major source of this pyruvate and lactate has seemed plausible hitherto. Fig. 13 shows however that throughout the rise in AV lactate concentrations, lactate was being taken up by the forearm tissues indicating that this elevation cannot be attributed to augmented peripheral lactate release. It must be postulated therefore that in normal subjects the rise is the consequence of increased glucose uptake, its conversion to lactate and subsequent release of this lactate

into the circulation by tissues other than those in the periphery. Since the liver is the most likely site of such lactate release, it may be concluded that (1) the timing and height of the lactate peak are a reflection of the promptness and magnitude of hepatic glucose uptake after glucose loading and (2) it is the change in hepatic rather than peripheral lactate balance which is the primary determinant of the shape of the lactate response curve.

The present findings are in excellent agreement with those of several other workers. Thus Cherry and Crandall (1937) showed that lactate was released by the liver after oral glucose loading in normal dogs and proposed that this lactate was then taken up by other tissues. Gordon and Craigie (1960) showed that net splanchnic production of pyruvate resulted after intravenous glucose administration in normal subjects. In diabetics Butterfield and Whichelow (1965) have verified that the large increases in blood pyruvate concentrations which result after the administration of glucose and insulin but not glucose alone (Fry and Butterfield, 1962), are associated with pyruvate release by the liver and pyruvate uptake by peripheral tissues. More recently both Craig (1966) and Woods and Krebs (1971) have confirmed that livers of well-fed rats release lactate when perfused with glucose, while Felig and Wahren (1971) have demonstrated the initiation of hepatic lactate release after glucose infusion in man. Finally, the changing pattern of lactate balance across the forearm during the GTTs (Fig. 13) is entirely consistent with the findings of many workers that skeletal muscle may both take up and release lactate (Omachi and Lifson, 1956; Carlson and Pernow, 1959; Harris et al., 1962; Stainsby and Welch, 1966; Freyschuss and Strandell, 1967) and that the direction of lactate transfer across the cell membrane is determined predominantly by the arterial lactate concentration (Jorfeldt, 1970).

It is tempting to speculate that following a glucose load, the

hepatic release of lactate and presumably, pyruvate (Fry and Butterfield, 1962; Butterfield and Whichelow, 1965), represents a mechanism, hitherto unrecognised, by which the concomitant assimilation of glucose by peripheral tissues is potentiated. Thus recent reports suggest that lactate may augment insulin-stimulated glycogenesis from glucose in skeletal muscle (Moorthy and Gould, 1969) while in adipose tissue lactate may inhibit lipolysis independently of insulin (Issekutz and Miller, 1962; Issekutz et al., 1965; Dieterle et al., 1970). Interestingly, Lang et al. (1954) suggested in dogs that maximal peripheral glucose assimilation after glucose loading is not dependent on insulin alone but "upon the release of some 'humoral' agent or influence by the liver" and in the light of the present findings, it is conceivable that lactate could be this humoral agent.

Fig. 14 shows that in the periods 0-15 and 15-30 min after glucose ingestion, glucose entry into the general circulation was substantial but was thereafter strikingly reduced for the remainder of the GTT. Since ingested glucose can reach the peripheral circulation only via the hepatic veins and since after absorption the liver is the first organ to be perfused by the glucose, it follows that any increase in hepatic glucose uptake will reduce simultaneously the amount of glucose available for subsequent entry into the general circulation. Thus the reduction in the rate of glucose entry seen after 30 min (Fig. 14) would seem to be a manifestation of rapidly increasing hepatic uptake of the absorbed glucose load. Indeed, during the 30-45 min period as against the preceding 15 min, the decrement in glucose reaching the general circulation was 7.1 g whereas the concomitant increment in peripheral glucose uptake was only 1.1 g. In the light of the current proposals regarding the mechanism of the lactate response, this is consistent with the continuing elevation in plasma lactate concentrations observed between 30-60 min (Fig. 13).

These proposals are in good agreement with the findings of Cherry and Crandall (1937) and Jeffcoate and Moody (1969) since the former workers (Cherry and Crandall, 1937) reported that hepatic glucose uptake in dogs after oral glucose loading was greatest only after 30 min while the latter (Jeffcoate and Moody, 1969) demonstrated that maximum rates of increase in liver glycogen levels during GTTs in rats were reached 20-60 min after glucose administration. It may be concluded, therefore, that peak rates of hepatic glucose uptake are achieved only some 30 min or more after glucose ingestions and that, in normal subjects, it is this continuing rise in hepatic uptake which is the major factor aborting any further increase in peripheral plasma glucose concentrations after this time (Fig. 9).

It has been noted above that the rise in peripheral glucose uptake is fairly evenly distributed during the 180 min of the GTT. In terms of the glucose tolerance curve as a whole, therefore, it would appear that while increased peripheral glucose consumption serves to minimise the increment in glucose concentrations throughout the test, it is primarily the hepatic response which determines the shape of the curve.

In peripheral tissues, as in the liver, there is a delay before the maximal response is achieved. Fig. 9 shows that although peak glucose and insulin concentrations were reached at 30 min, peak rates of peripheral glucose utilisation were achieved only at 60 min. The reason for this delay before the peak responses are reached in liver and peripheral tissues is probably the requirement by both these tissues for a period of exposure to insulin before the effects of hormone action become fully manifest. There is general agreement that the tissue binding of insulin is a necessary prerequisite for the initiation of any metabolic change and precedes by a latent period (phase of tissue "priming" or "insulinisation") the increase in tissue

permeability to glucose (Stadie et al., 1949; Stadie et al., 1952; Rafaelson et al., 1965; Wohtmann and Narahara, 1966; Gould and Chaudry, 1970). In liver, tissue insulin binding has been well demonstrated and amounts to some 50-80% of the insulin perfusing the organ in one circulation (Madison et al., 1959; Samols and Ryder, 1961), but little information is available regarding peripheral insulin uptake in man (Butterfield,et al., 1963).

While the results are insufficient to allow the formulation of a mathematical relationship between tissue insulin-binding and tissue glucose uptake, they are compatible with certain accepted concepts of insulin action. Thus, the maintenance of high rates of FGU between 30-120 min (Fig. 12) despite the sharp decline in concomitant forearm insulin uptake after 30 min could be evidence for the sustained localised effect of tissue-bound hormone (Rafaelson et al., 1965); the early fall-off in tissue insulin uptake in the face of relatively sustained AV insulin concentrations between 30-120 min is consistent with a progressive diminution in tissue affinity for insulin as saturation of available binding sites is achieved. Furthermore, the correlation of forearm insulin uptake with concomitant AV insulin levels could be evidence of the concentration-dependence of the quantity of hormone which ultimately become tissue-bound (Garrett et al., 1966). One cannot be certain, however, that forearm insulin uptake has not been overestimated at 30 min in view of the rapidly rising insulin concentration at this time and the lack of additional observations shortly before or after 30 min (Fig. 12). Further detailed data are required, therefore, to substantiate the present findings.

It is well known that the insulin response to glucose loading in normal subjects varies over a wide range and that each individual has his or her own particular level of response; it is not clear however, which factor primarily determines the absolute level of the

response. Conceivably, the degree of peripheral insulin responsiveness peculiar to each individual is this basic factor and that over the years the islets adapt to or "learn" that level of insulin secretion which is required in that individual in order to maintain glucose homeostasis after glucose ingestion. If this were so, it would be anticipated that the level of insulin response to glucose loading would vary inversely with the level of peripheral insulin responsiveness. The inverse correlation shown in Fig. 10 could be evidence for such an hypothesis.

SUMMARY

Total forearm glucose utilisation was determined during 100 g GTTs in 25 normal volunteers by a simple technique involving the sampling of AV and MV blood and the measurement of forearm blood flow with the mercury-in-rubber strain gauge. In addition, the concomitant balance of insulin, growth hormone and lactate across the forearm was studied in 13 subjects.

Plasma glucose concentrations rose steeply during the first 30 min after glucose loading but fell progressively thereafter. The increment in FGU during the 180 min following glucose ingestion amounted to 74 mg/100 ml forearm; it may be calculated that increased peripheral glucose utilisation accounted for the disposal of 43% of the 100 g load and that during the GTT some 58 g of extra glucose reached the peripheral circulation.

Serum insulin concentrations in MV blood rose steeply after glucose loading while growth hormone levels were reduced. Additional observations in 13 subjects showed that MV insulin levels remained significantly lower than corresponding AV concentrations between 30–150 min, suggesting that insulin was being continually removed by the

forearm tissues during this time. On the same serum samples, however, significant AV-MV differences were not detected in growth hormone levels. Both the AV-MV insulin differences and the rates of forearm insulin uptake were significantly correlated with corresponding AV insulin concentrations during the test.

Plasma lactate concentrations rose immediately after glucose loading, reaching a peak at 60 min and declining thereafter. The initial elevation was associated with lactate uptake by the forearm and the subsequent fall with lactate release, suggesting that peripheral lactate metabolism has little or no influence on the shape of the lactate response curve. It is suggested that this early rise in lactate concentrations is the result of increased hepatic lactate production and that the timing and height of the lactate peak reflects the pattern of enhanced hepatic glucose utilisation after oral glucose loading.

The results suggest that the disposition of a 100 g oral glucose load is accounted for mainly by hepatic glucose conservation rather than peripheral uptake. Thus while peripheral glucose consumption serves to minimise the increment in glucose concentrations throughout the GTT, it would appear to the the hepatic response which is the major determinant of the shape of the oral glucose tolerance curve.

CHAPTER 6

THE INFLUENCE OF A LOW CARBOHYDRATE DIET ON THE RESPONSE TO GLUCOSE LOADING IN NORMAL SUBJECTS

INTRODUCTION

It has long been known that starvation or the restriction of dietary carbohydrate in animals or man results in a marked decrease in the body's ability to utilize carbohydrate. Ingestion of a test dose of glucose under these conditions produces hyperglycaemia, glycosuria, and a marked delay in clearing the blood of absorbed glucose. In 1889 Hofmeister found that if he gave a carbohydrate meal to normal dogs that had been starving previously, glycosuria resulted - a phenomenon which he called "hunger diabetes". Since that time a number of investigators, working with several species of animals as well as human subjects, have confirmed and extended these findings. Bang (1913), Staub (1922), Goldblatt (1925), du Vigneaud and Karr (1925) and others have all found hyperglycaemia, glycosuria and delayed glucose removal from the blood stream when carbohydrate was fed after a period of starvation; similar responses have been documented following a low carbohydrate diet by Southwood (1923), Greenwald et al. (1924), Sweeny (1927), Malmross (1928) and Tolstoi (1929). Further documentation of this phenomenon was provided by Kageura (1922), Aldersberg and Forges (1926), McClellan and Wardlaw (1932), Hinsworth (1935) and McCullagh and Johnstone (1935).

Dann and Chambers (1930) observed that a few days of carbohydrate feeding were sufficient to restore to normal the decreased utilisation of carbohydrate induced by prolonged starvation. Sweeny (1927) demonstrated that a single preliminary dose of glucose did not succeed in counteracting the effects of an abnormal antecedent diet.

Much excellent work dealing with the mechanism of this response was done by Dann and Chambers (1932, 1933), Chambers (1938) and Haist

et al. (1939). Little certain knowledge emerged other than that the decrease in carbohydrate tolerance could be completely eliminated by a relatively short period of high carbohydrate feeding.

Conn (1940) studied the question further and emphasised the importance of the preparatory diet in the diagnosis of mild or questionable cases of diabetes where any influence of the previous diet on glucose tolerance must be eliminated. He recommended a standard preparatory diet for at least three days prior to the performance of the test; the diet should contain 300 g carbohydrate, 80 g protein and, at least, maintain calories. He suggested that when one encountered a diabetic type glucose tolerance curve with a normal or near normal fasting blood sugar value the test should be repeated after adequate dietary preparation since the greater effort and time entailed in the latter does not justify errors in diagnosis due to lack of such procedure. Conn (1940) noted that diabetics who have taken the standard preparatory diet for 3-5 days are not harmed in any way since the temporary increase in glycosuria disappears shortly after the test diet is discontinued.

The development in nondiabetics of impaired glucose tolerance following starvation (starvation diabetes) or a low carbohydrate diet (dietary diabetes) has been documented even more recently by several authors (van Itallie and Shull, 1957; Wilkerson et al., 1960; Hales and Randle, 1963; Unger et al., 1963; Beck et al., 1964; Benson and Yalow, 1965; Sussman, 1966; Tzagournis and Skillman, 1970; Jackson et al., 1971), but the mechanism of this impairment remains poorly understood. Hales and Randle (1963) suggested that the basic abnormality was a diminution in peripheral glucose utilisation consequent on an increase in peripheral insulin resistance, while Tzagounis and Skillman (1970) proposed that delayed and/or deficient

insulin secretion was the major underlying disturbance; on the other hand, the findings of Hornichter and Brown (1969) suggest that in this context the importance of decreased hepatic glucose utilisation cannot be overlooked. From these and other studies, however, no concept may be formulated of the relative importance of the above-mentioned factors in the pathogenesis of the reduction in glucose tolerance. Thus the conclusions reached were based largely on changes in circulating glucose and insulin concentrations which, in the absence of data on concomitant peripheral or hepatic glucose uptake, are open to several alternative and equally acceptable interpretations.

The present aim, therefore, was an assessment of the role of changes in peripheral glucose uptake in the production of dietary diabetes by studying peripheral glucose utilisation directly in conjunction with glucose tolerance. This was achieved by measuring changes in forearm metabolism during GTTs in normal volunteers before and after a low carbohydrate diet, where each subject acted as his own control.

SUBJECTS AND METHODS

13 of the normal male volunteers described in Chapter 5 were studied (Table 21). In each subject a GTT was carried out before and after a low carbohydrate diet. Before the control GTT, each subject consumed at least 220 g carbohydrate daily for five days, but for seven days prior to the second GTT, a low calorie, low carbohydrate diet (see Chapter 4); the aim of restricting calories in addition to carbohydrate was the induction of significant ketonaemia as rapidly as possible. During this period, all subjects lived in the metabolic ward, but were ambulant and continued normal daily activities throughout.

TABLE 21

Clinical data

Subject	Age	Height	Body weight	Total weight loss
	yr	cm	kg	kg
A.Y.	23	173	67.0	6.2
C.K.	22	173	62.4	2.8
M.B.	22	186	72.9	4.0
D.M.	22	178	66.3	5.0
J.M.	20	177	76.5	6.5
R.M.	20	179	76.8	2.8
G.M.	21	175	76.4	3.9
J.R.	32	163	53.5	3.6
D.T.	21	183	74.0	6.4
A.P.	42	179	70.0	3.5
T.P.	47	176	73.9	4.5
G.T.	20	163	67.5	4.3
C.H.	23	176	72.0	6.0

RESULTS

(a) Response to glucose loading (Tables 22 and 23)

After carbohydrate restriction, basal glucose concentrations were reduced, glucose tolerance was impaired and the rise in serum insulin levels delayed (Fig. 15). The mean glucose and insulin responses after carbohydrate restriction differed significantly from control values at all points except those adjacent to the intersection of the corresponding curves, i.e. 30 min for each glucose curve and 60 and 75 min for the insulin curve (for the AV glucose curve - $p < .005$ at 15 and 180 min and $< .001$ at remaining points; for the MV glucose curve - $p < .005$ at 15, 60, 105 and 180 min and $< .001$ at remaining points; for the insulin curve - $p < .05$ at 45 min, $< .02$ at 0 and 180 min, $< .005$ at 135-165 min and $< .001$ at remaining points). Apart from an elevation at 165 min ($p < .05$), however, levels of FGU were unaffected by dietary change. Forearm blood flow during the GTT remained similarly unchanged, the results (mean ± SEM) being respectively 3.3 ± 0.2 and 3.0 ± 0.3 ml/100 ml forearm/min before and after carbohydrate restriction. Fig. 16 (see Appendix, Table 5) illustrates the influence of carbohydrate restriction on the interrelationship between the concentrations of glucose and insulin and FGU when the incremental area is used as the index of response. Both the levels and patterns of the glucose and insulin responses were significantly altered by carbohydrate restriction. Thus, the elevation in glucose concentrations after the low carbohydrate diet was increased through ut and maximal during the second hour of the GTT. In association with the latter, a late but prolonged rise in insulin levels was observed and as a result, the overall (0-180 min) insulin response was increased. FGU was not diminished at any phase

TABLE 22

Mean response following 100 g oral glucose load after normal diet in 13 normal subjects

	Minutes						
	0	15	30	45	60	75	90
Glucose AV* mg/100 ml	86 ± 1**	127 ± 5	169 ± 4	164 ± 6	152 ± 6	142 ± 7	133 ± 7
MV §	85 ± 1	105 ± 3	133 ± 5	126 ± 7	112 ± 7	106 ± 8	104 ± 7
Glucose uptake mg/100 ml forearm/min	.026 ± .008	.332 ± .073	.565 ± .064	.616 ± .076	.714 ± .069	.685 ± .073	.595 ± .064
Insulin μU/ml	10 ± 1	41 ± 6	84 ± 8	77 ± 9	73 ± 9	70 ± 9	66 ± 10
FFA μmol/l	437 ± 2	–	329 ± 7	–	211 ± 8	–	187 ± 4
Growth hormone ng/ml	2.4 ± 0.5	–	1.6 ± 0.3	–	1.1 ± 0.1	–	1.8 ± 0.5
Blood flow ml/100 ml forearm/min	2.8 ± 0.2	2.4 ± 0.2	2.7 ± 0.3	2.9 ± 0.3	3.1 ± 0.3	3.2 ± 0.3	3.4 ± 0.2

* Arterialised venous
** Standard error of mean
§ Mixed venous

TABLE 22 Cont.

Mean response following 100 g oral glucose load after normal diet in 13 normal subjects

	Minutes					
	105	120	135	150	165	180
Glucose AV* mg/100 ml	126 ± 5**	119 ± 5	106 ± 5	107 ± 6	104 ± 7	100 ± 6
MV §	98 ± 6	95 ± 5	86 ± 5	86 ± 5	88 ± 6	87 ± 5
Glucose uptake mg/100 ml forearm/min	.566 ± .047	.500 ± .044	.421 ± .050	.456 ± .048	.392 ± .049	.296 ± .044
Insulin μU/ml	59 ± 8	52 ± 7	41 ± 5	42 ± 6	38 ± 6	32 ± 5
FFA μmol/l	–	174 ± 3	–	173 ± 2	–	197 ± 9
Growth hormone ng/ml	–	3.4 ± 1.3	–	3.5 ± 2.1	–	5.5 ± 2.5
Blood flow ml/100 ml forearm/min	3.5 ± 0.2	3.5 ± 0.3	3.5 ± 0.2	3.7 ± 0.3	4.2 ± 0.3	4.1 ± 0.4

* Arterialised venous
** Standard error of mean
§ Mixed venous

TABLE 23

Mean response following 100 g oral glucose load after carbohydrate restriction in 13 normal subjects

	Minutes						
	0	15	30	45	60	75	90
Glucose AV* mg/100 ml	67 ± 2**	109 ± 3	159 ± 5	195 ± 5	198 ± 7	187 ± 10	182 ± 10
p§	<.001	<.005	N.S.	<.001	<.001	<.001	<.001
MV‡	67 ± 2	89 ± 3	127 ± 5	152 ± 5	160 ± 6	149 ± 10	149 ± 10
p	<.001	<.005	N.S.	<.001	<.005	<.001	<.001
Glucose uptake mg/100 ml forearm/min	.034 ± .010	.277 ± .032	.458 ± .045	.614 ± .064	.596 ± .065	..645 ± .087	.551 ± .072
p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Insulin μU/ml	7 ± 1	22 ± 3	43 ± 5	65 ± 6	79 ± 8	78 ± 9	86 ± 9
p	<.02	<.001	<.001	<.05	N.S.	N.S.	N.S.
FFA μmol/l	1047 ± 100	–	797 ± 54	–	454 ± 36	–	360 ± 36
p	<.001		<.001		<.001		<.001
Growth hormone ng/ml	6.1 ± 2	–	3.0 ± 0.6	–	2.6 ± 0.7	–	4.1 ± 1.7
p	N.S.		<.05		N.S.		N.S.
Blood flow ml/100 ml forearm/min	3.3 ± 0.3	2.3 ± 0.3	2.5 ± 0.2	2.6 ± 0.4	2.8 ± 0.4	2.8 ± 0.3	3.0 ± 0.4
p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Arterialised venous
** Standard error of mean
§ Significance of difference between means before and after carbohydrate restriction
‡ Mixed venous

TABLE 23 Cont.

Mean response following 100 g oral glucose load after carbohydrate restriction in 13 normal subjects

		Minutes					
		105	120	135	150	165	180
Glucose ng/100 ml	AV*	175 ± 10 **	168 ± 9	160 ± 9	154 ± 9	145 ± 10	134 ± 11
	p §	<.001	<.001	<.001	<.001	<.001	<.005
	MV †	139 ± 11	133 ± 9	127 ± 10	125 ± 10	116 ± 10	112 ± 11
	p	<.005	<.001	<.001	<.001	<.001	<.005
Glucose uptake ng/100 ml forearm/min		.617 ± .078	.655 ± .074	.590 ± .078	.576 ± .074	.577 ± .095	.447 ± .082
	p	N.S.	N.S.	N.S.	N.S.	<.05	N.S.
Insulin µU/ml		92 ± 2	89 ± 0	75 ± 1	75 ± 9	56 ± 7	50 ± 8
	p	<.001	<.001	<.005	<.005	<.005	<.02
FFA µmol/l		–	341 ± 28	–	359 ± 30	–	490 ± 55
	p		<.001		<.001		<.001
Growth hormone ng/ml		–	5.6 ± 2.1	–	4.6 ± 1.7	–	3.4 ± 0.9
	p		N.S.		N.S.		N.S.
Blood flow ml/100 ml forearm/min		3.0 ± 0.4	3.4 ± 0.5	3.2 ± 0.4	3.3 ± 0.4	3.5 ± 0.4	3.6 ± 0.4
	p	N.S.	N.S.	N.S.	N.S.	<.05	N.S.

* Arterialised venous
** Standard error of mean
§ Significance of difference between mean before and after carbohydrate restriction
† Mixed venous

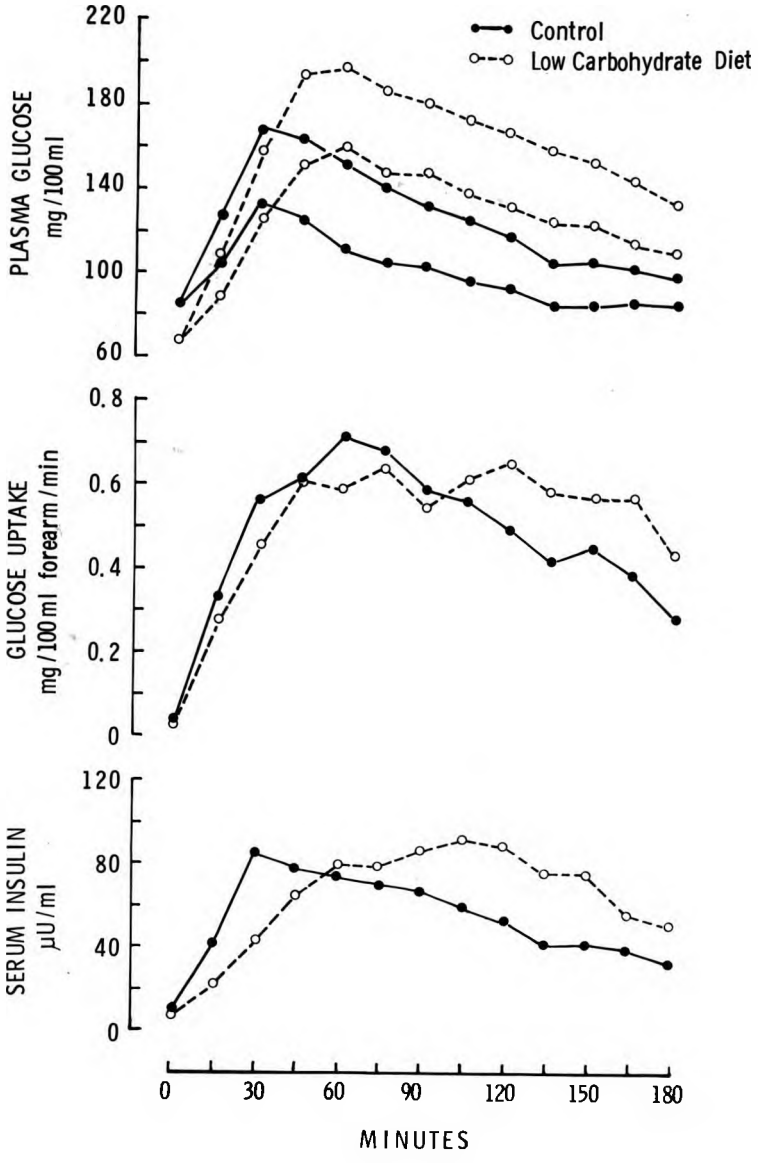

FIG. 15 Influence of carbohydrate restriction on the mean response following 100 g oral glucose load in 13 normal subjects. The upper glucose curves denote levels in arterialised venous plasma, the lower curves levels in mixed venous plasma

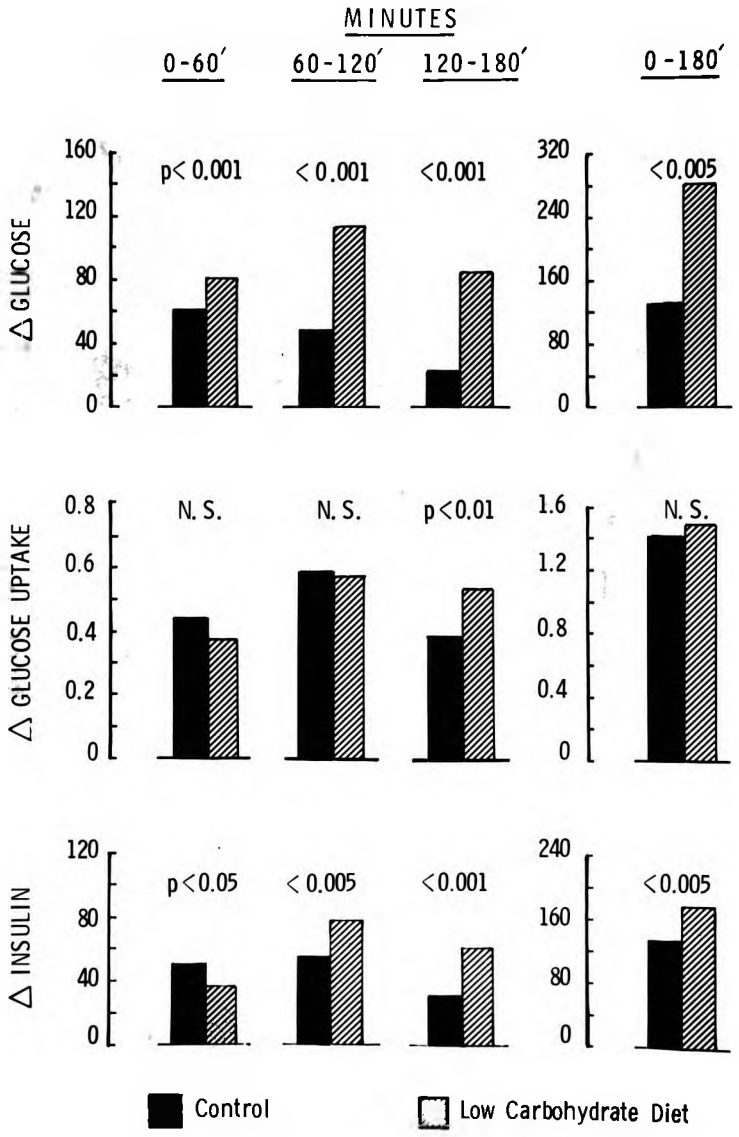

FIG. 16 Influence of carbohydrate restriction on the mean response following 100 g oral glucose load expressed as the incremental area under the curve (Δ) in 13 normal subjects

of the GTT or in the GTT as a whole; on the contrary, after carbohydrate restriction, uptake was significantly increased between 120-180 min. Table 24 shows that while in the GTT as a whole, carbohydrate restriction decreases β-cell responsiveness to glucose (ΔInsulin/ΔGlucose) and reduces peripheral insulin sensitivity (ΔFGU/ΔInsulin), changes in the latter are variable at different phases within the GTT. This is probably explained by the questionable validity of expressing peripheral insulin resistance as ΔFGU/ΔInsulin since FGU is determined not only by the insulin response, but also by prevailing glucose concentrations which are not taken into account in this calculation.

The delayed rise in serum insulin concentrations after the low carbohydrate diet was not a universal response but occurred in 11 of the 13 subjects; despite the normally prompt rise in insulin concentrations in the two remaining volunteers (G.T. and G.M.), glucose tolerance was decreased in each subject. The data of one of these volunteers (G.T.) are shown in Fig. 17 (see Appendix, Table 7).

The relative importance of the extracellular concentrations of glucose and insulin in regulating FGU cannot be clearly defined from the results of GTTs since, in the latter, both are variables. Thus in order to dissociate the influence on FGU of insulin alone from that of hyperglycaemia in its own right regional intra-arterial insulin infusions, during which constant AV glucose levels were maintained, were carried out before and after carbohydrate restriction on each of two subjects (R.M. and D.T.). Fig. 18 (see Appendix, Table 8) shows that although the forearm tissues were exposed to similar insulin concentrations on each occasion, insulin-stimulated glucose uptake was reduced following a low carbohydrate diet in each volunteer.

TABLE 24

Analysis of glucose tolerance tests based on use of incremental areas (Δ)

	Minutes			
	0 - 60	60 - 120	120 - 180	0 - 180
ΔInsulin				
Glucose *				
Control	0.85 ± 0.09**	1.19 ± 0.13	1.54 ± 0.21 §	1.06 ± 0.10
Low carbohydrate diet	0.47 ± 0.06	0.72 ± 0.08	0.76 ± 0.07	0.66 ± 0.06
p"	< .001	< .001	< .005	< .001
ΔFGU				
Insulin				
Control	9.76 ± 1.25	12.94 ± 1.50	15.82 ± 2.39	11.68 ± 1.20
Low carbohydrate diet	13.35 ± 2.68	7.79 ± 0.99	9.40 ± 1.13	8.73 ± 0.94
p	N.S.	< .005	< .01	< .01

* Calculated from concentrations in arterialised-venous plasma
n = 13

** Standard error of mean

§ n = 12 for this particular set of observations

" Significance of difference

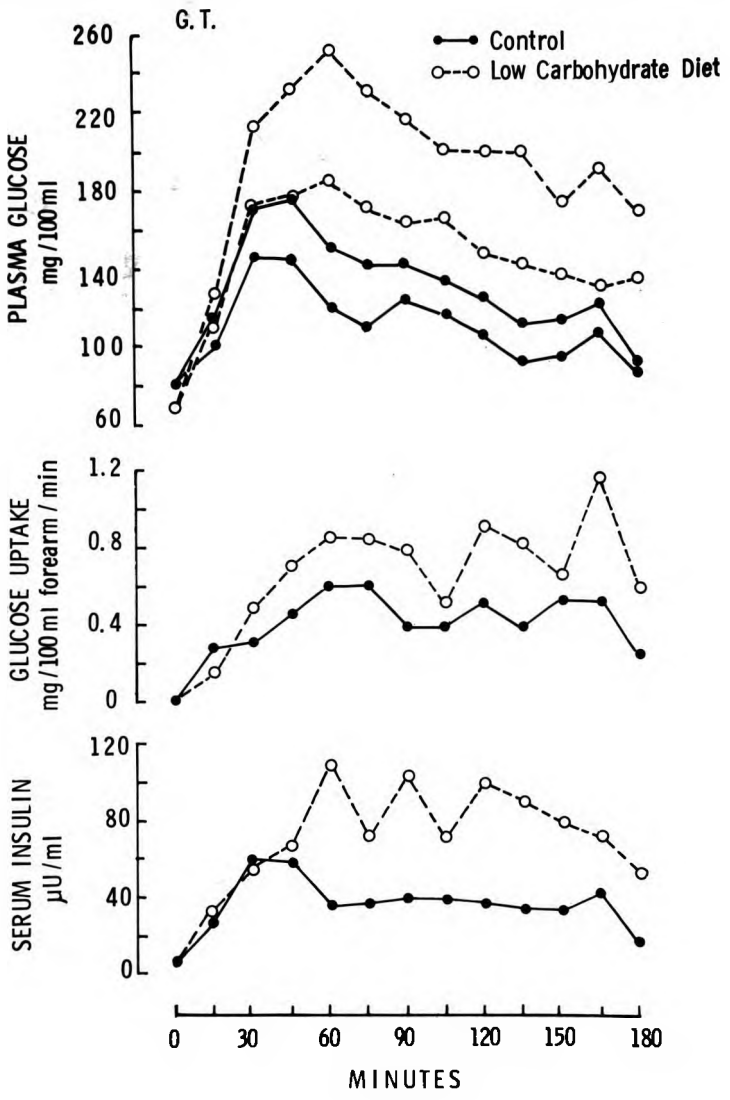

FIG. 17 Influence of carbohydrate restriction on the response following 100 g oral glucose load in a normal subject (G.T.)

The lactate responses during the control GTT have been described in Chapter 5 (Fig. 13; Table 18) and were influenced in several respects by carbohydrate restriction (Fig. 19; Table 25). Firstly basal AV lactate levels were significantly reduced ($p < .05$) while MV levels were not; since in their study Kreisberg et al. (1970) sampled venous blood only, these observations probably explain the failure of these workers to detect any change in lactate concentrations

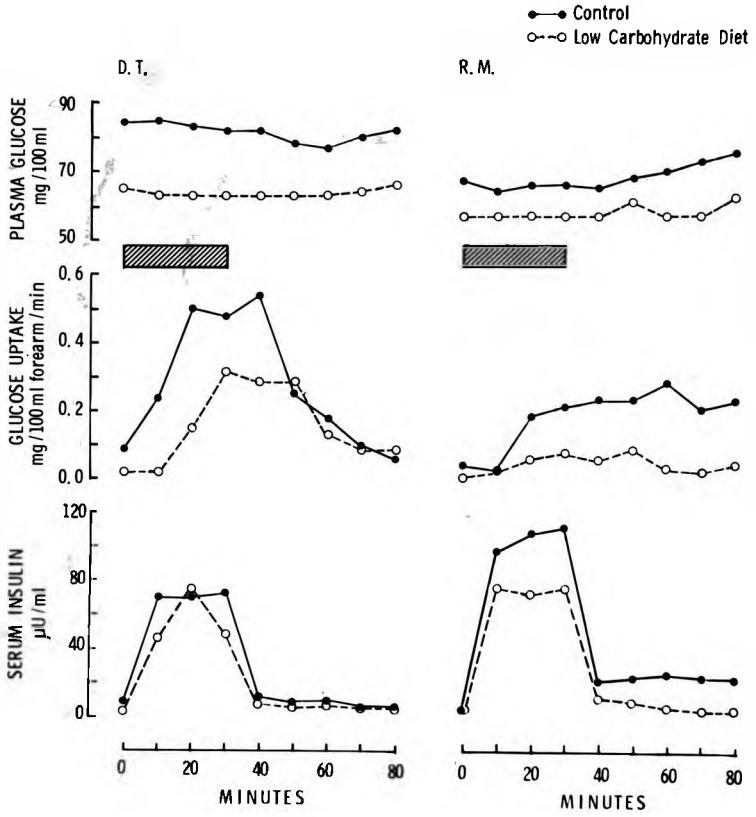

FIG. 18 Influence of carbohydrate restriction on the changes in forearm glucose utilisation in response to regional intra-arterial insulin infusion in normal subjects

after starvation. Secondly, whereas under control conditions lactate was neither taken up nor released by forearm tissues basally, carbohydrate restriction resulted in a significant increase ($p<.02$) in basal lactate production to a level of .172 mmol/100 ml forearm/min. Similar findings were reported by Zierler and Rabinowitz (1963) but not, for reasons which remain obscure, by Owen and Reichard (1971). Thirdly, although on the control diet glucose loading induced an immediate rise in lactate concentrations to a peak at 60 min, levels were initially reduced after carbohydrate restriction ($p<.001$ at 30 min) and though increasing subsequently, the rise was slow and

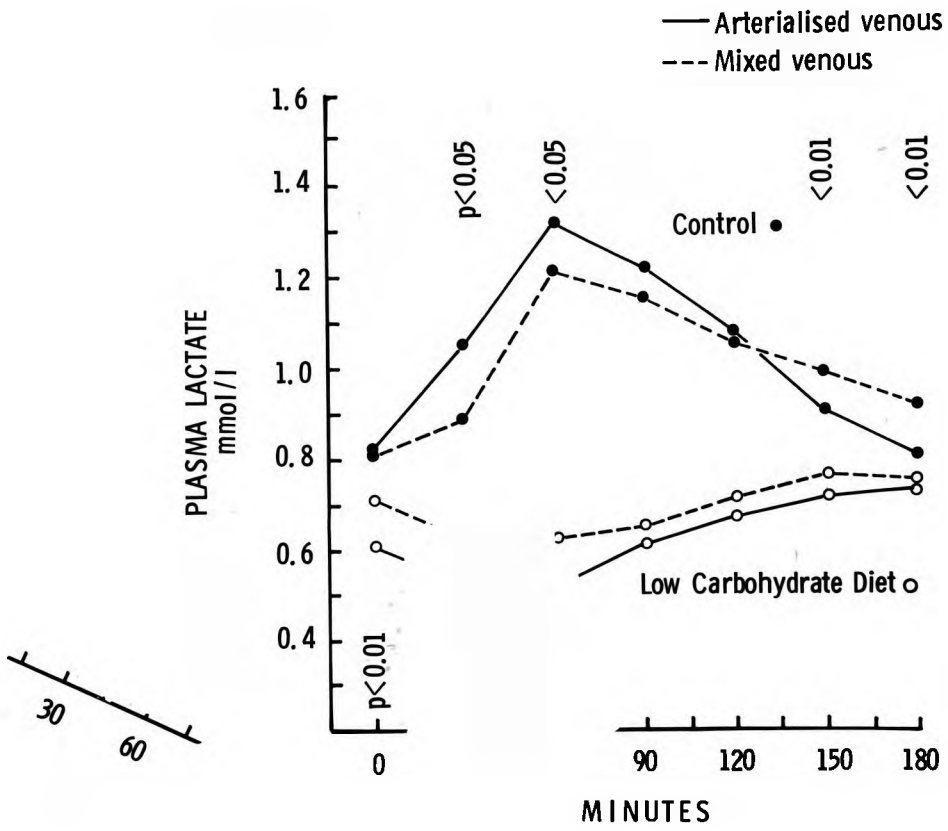

FIG. 19 Influence of carbohydrate restriction on the mean changes in lactate concentrations following 100 g oral glucose load in 11 normal subjects

considerably delayed. Finally, whereas lactate was taken up by forearm tissues during the first 60 min of the control GTT, lactate release continued throughout the first hour of the GTT following the low carbohydrate diet.

On a normal carbohydrate diet, neither acetoactetate nor β-hydroxybutyrate were detected in blood but following carbohydrate restriction, marked elevations in basal FFA, β-hydroxybutyrate and acetoacetate concentrations were observed (Fig. 20; Tables 23 and 26). The reduction in FFA concentrations 60 min after glucose ingestion (Bagdade et al., 1969) was unaffected by the dietary change, being

TABLE 25

Mean lactate concentrations (mmol/l) in arterialised venous (AV) and mixed venous (MV) plasma following 100 g oral glucose load in 11 normal subjects

	Minutes						
	0	30	60	90	120	150	180
Control							
AV	0.82 ± 0.7*	1.05 ± .11	1.31 ± .11	1.21 ± .10	1.07 ± .08	0.90 ± .07	0.80 ± .06
MV	0.81 ± .07	0.89 ± .06	1.21 ± .09	1.15 ± .07	1.05 ± .09	0.98 ± .07	0.91 ± .07
p**	N.S.	<.05	<.05	N.S.	N.S.	<.01	<.01
Low carbohydrate diet							
AV	0.61 ± .03	0.52 ± .04	0.52 ± .04	0.61 ± .04	0.67 ± .04	0.71 ± .06	0.72 ± .06
MV	0.71 ± .05	0.62 ± .05	0.62 ± .05	0.65 ± .05	0.71 ± .04	0.76 ± .05	0.73 ± .06
p	<.01	<.001	<.001	N.S.	N.S.	N.S.	N.S.

* Standard error of mean

** Significance of difference between means before and after carbohydrate restriction

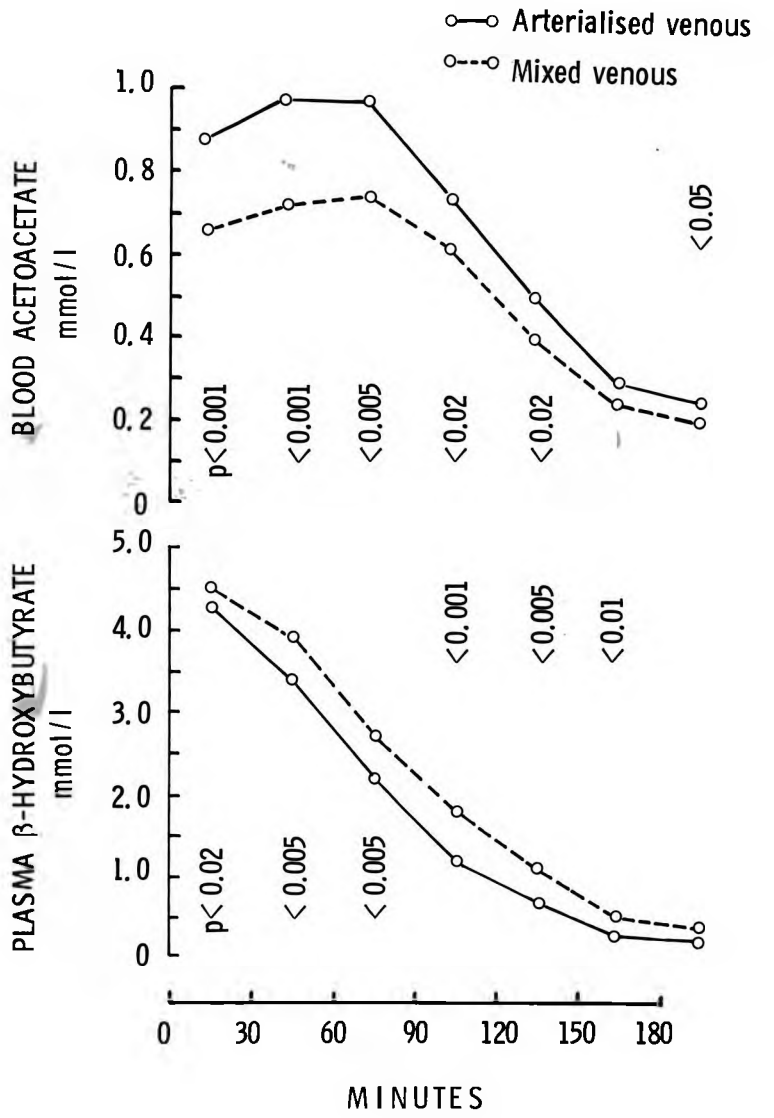

FIG. 20 Mean changes in acetoacetate (n = 7) and β-hydroxybutyrate (n = 13) concentrations following 100 g oral glucose load after carbohydrate restriction in normal subjects

TABLE 26

Mean acetoacetate and β-hydroxybutyrate concentrations (mmol/l) in arterialised venous (AV) and mixed venous (MV) blood following 100 g oral glucose load after carbohydrate restriction in normal subjects

	Minutes						
	0	30	60	90	120	150	180
Blood acetoacetate							
AV	.88 ± .13*	.97 ± .13	.96 ± .19	.72 ± .16	.48 ± .13	.27 ± .10	.22 ± .07
MV	.66 ± .11	.72 ± .11	.73 ± .15	.60 ± .16	.38 ± .11	.22 ± .08	.17 ± .05
p**	<.001	<.001	<.005	<.02	<.02	N.S.	<.05
n	7	7	7	7	7	5	5
Plasma β-hydroxybutyrate							
AV	4.3 ± 0.6	3.4 ± 0.5	2.2 ± 0.4	1.2 ± 0.3	0.7 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
MV	4.5 ± 0.6	3.9 ± 0.5	2.7 ± 0.4	1.8 ± 0.4	1.1 ± 0.3	0.5 ± 0.2	0.3 ± 0.1
p	<.02	<.005	<.005	<.001	<.005	<.01	N.S.
n	13	13	13	13	12	9	7

* Standard error of mean

** Significance of difference between means

51.8% before and 56.6% after the low carbohydrate diet. Following carbohydrate restriction, a clear dissociation was observed between the acetoacetate and β-hydroxybutyrate responses during the GTTs (Fig. 20). Thus β-hydroxybutyrate concentrations fell immediately ($p < .001$ at 30 min) and continued to decline throughout the GTT; acetoacetate levels on the other hand, rose imitially ($p < .005$ at 30 min) and fell only during the second hour of the test. Both basally and after glucose loading, acetoacetate was taken up by the forearm while β-hydroxybutyrate was released. In those subjects in whom blood acetoacetate was determined, the uptake of this metabolite by the forearm tissues was insufficient to account for the concomitant β-hydroxybutyrate release (Fig. 21; see Appendix. Table 9); forearm acetoacetate uptake decreased progressively during the GTT while β-hydroxybutyrate release was initially increased and remained elevated except during the final 30 min of the test. It has been confirmed in separate experiments in each of two subjects (T.P. and J.R.) both before (Chapter 5, cf. Tables 18 and 19) and after carbohydrate restriction (Table 27; cf. Tables 25 and 26) that the trends found in the balance of lactate and ketone bodies across the forearm are similar whether arterial or AV blood is sampled and whether, with respect to lactate and β-hydroxybutyrate, estimations are performed on plasma or whole blood precipitated immediately on collection in ice-cold perchloric acid (0.6M). The results of one of these experiments performed after carbohydrate restriction is shown in Fig. 22. Basally, an increase in mean serum growth hormone levels was found after the low carbohydrate diet. but this elevation was not statistically significant (Table 23). After carbohydrate restriction, no correlation was found between basal concentrations of growth hormone and those of FFA or β-hydroxybutyrate, neither were any of these values correlated with the degree of glucose intolerance induced

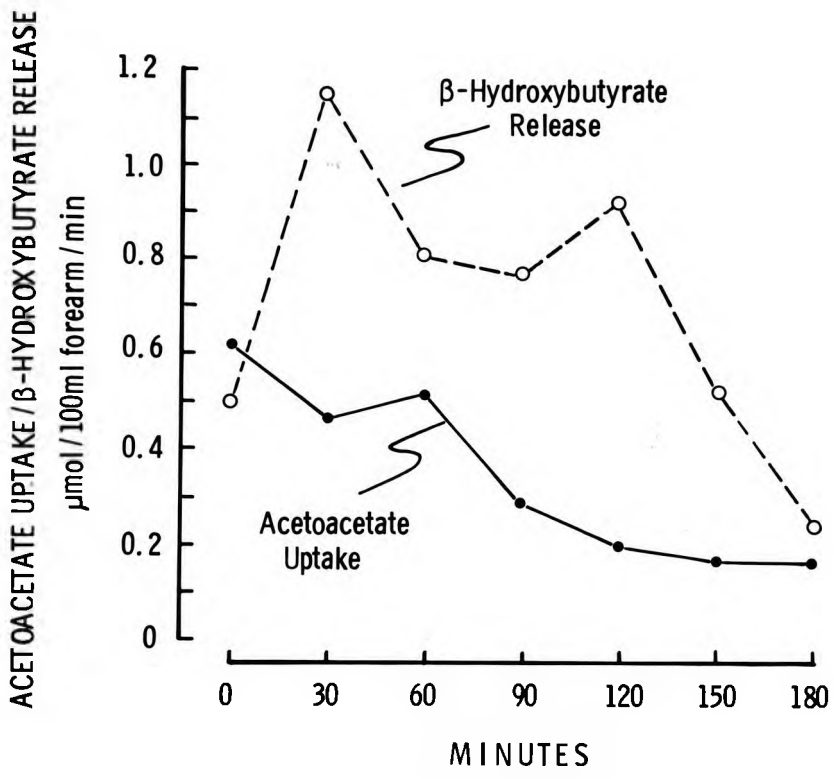

FIG. 21 Comparison of acetoacetate uptake and concomitant β-hydroxybutyrate release by the forearm after glucose loading in 7 normal subjects

by the low carbohydrate diet (expressed as the percent change in the incremental area under the AV glucose curve). The serum potassium concentrations were unaltered by carbohydrate restriction in 5 subjects (Table 28) as was the half-life of exogenous insulin in 3 subjects (Fig. 23; Table 29 and see Appendix, Table 10) all of whom developed marked glucose intolerance.

(b) Fate of the ingested glucose load

On the basis of the present observations and the assumptions previously detailed (Chapter 5), the disposition of the glucose load

TABLE 27

Lactate, acetoacetate and β-hydroxybutyrate concentrations (mmol/l) in arterial (A) and mixed venous (MV) blood following 100 g oral glucose load after carbohydrate restriction

Subject			Minutes												
			0	15	30	45	60	75	90	105	120	135	150	165	180
J.R.	Lactate	A	0.20	0.22	0.18	0.16	0.15	0.14	0.15	0.16	0.24	0.17	0.22	0.25	0.49
		MV	0.43	0.40	0.41	0.28	0.25	0.23	0.23	0.24	0.32	0.31	0.38	0.48	0.52
	Acetoacetate	A	1.52	1.67	2.08	2.22	2.20	2.03	1.94	1.60	1.25	0.93	0.60	-	0.40
		MV	1.13	1.15	1.33	1.70	1.50	1.42	1.28	1.10	0.90	0.70	0.50	-	0.35
	β-hydroxybutyrate	A	4.7	4.6	4.3	4.0	3.2	2.3	1.6	1.3	0.7	0.5	0.4	-	0.4
		MV	5.8	5.9	5.0	4.7	3.9	3.0	2.4	2.2	1.7	1.6	1.5	-	0.8
T.P.	Lactate	A	0.29	-	0.23	-	0.31	-	0.22	-	0.30	-	0.31	-	0.44
		MV	0.53	-	0.35	-	0.36	-	0.43	-	0.43	-	0.46	-	0.46
	Acetoacetate	A	1.12	-	1.24	-	1.38	-	1.15	-	0.85	-	0.60	-	0.50
		MV	0.77	-	0.75	-	0.90	-	0.92	-	0.65	-	0.35	-	0.32
	β-hydroxybutyrate	A	4.1	-	3.6	-	2.8	-	1.7	-	0.8	-	0.7	-	0.6
		MV	4.2	-	3.9	-	2.9	-	2.5	-	1.6	-	0.9	-	0.7

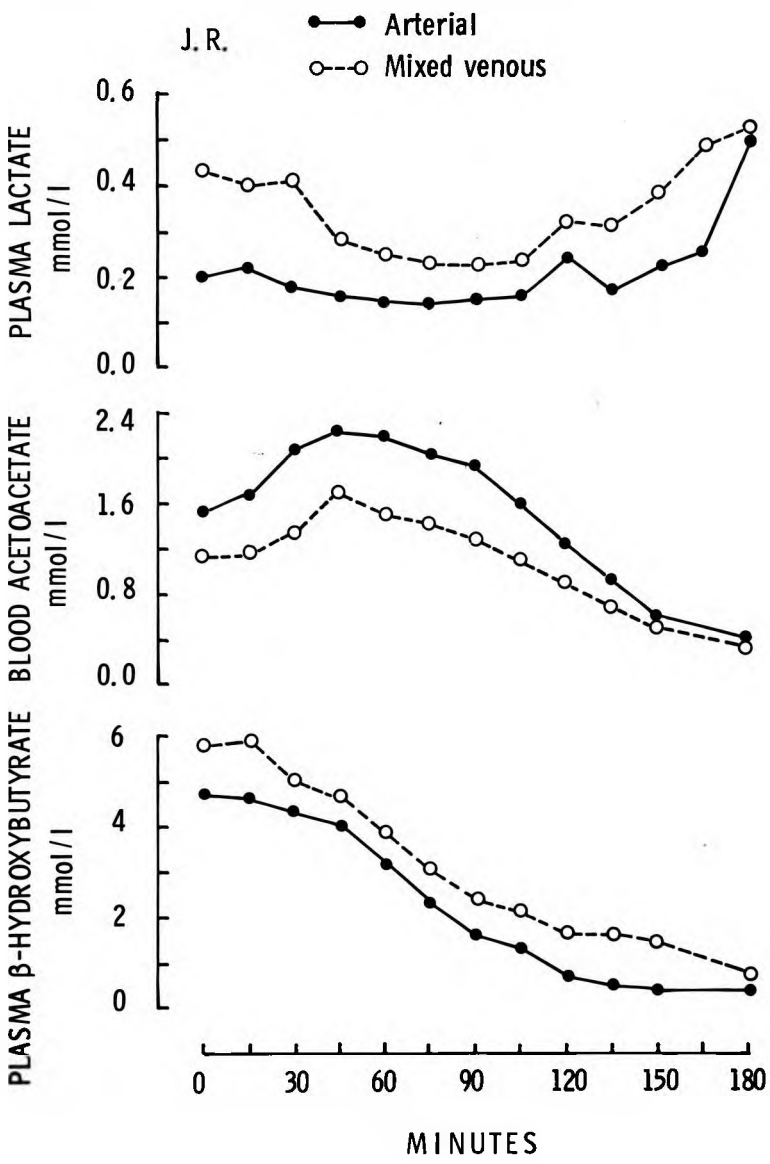

FIG. 22 Changes in lactate, acetoacetate and β-hydroxybutyrate concentrations following 100 g oral glucose load in a normal subject (J.R.) after carbohydrate restriction

TABLE 28

Serum potassium (mEq/l) before and after carbohydrate restriction

Subject	Before	After
G.M.	4.0	3.9
J.M.	3.9	3.9
J.R.	4.3	4.2
T.P.	4.3	4.4
G.T.	3.8	4.3
A.P.	4.3	4.2

in the body before and after the low carbohydrate diet has been calculated (Table 30). Although an estimate for overall hepatic glucose conservation after carbohydrate restriction may be derived, the relative contributions to the latter of decreased glucose output and increased glucose uptake (Mebane et al., 1961; Bishop et al., 1965) cannot be calculated. Urinary glucose loss was not estimated but comparison of the individual AV glucose concentrations with the renal threshold for plasma glucose, viz. 180-200 mg/100 ml (Pitts, 1968), suggests that a mean loss greater than 1 g during the GTTs would have been unlikely. The major changes in the disposition of the glucose load induced by carbohydrate restrictions were (1) a reduction in total hepatic glucose conservation and (2) an increase in the amount of glucose remaining within the glucose space 180 min after ingestion (Table 30).

Fig. 24 (see Appendix, Table 11) illustrates the increment in

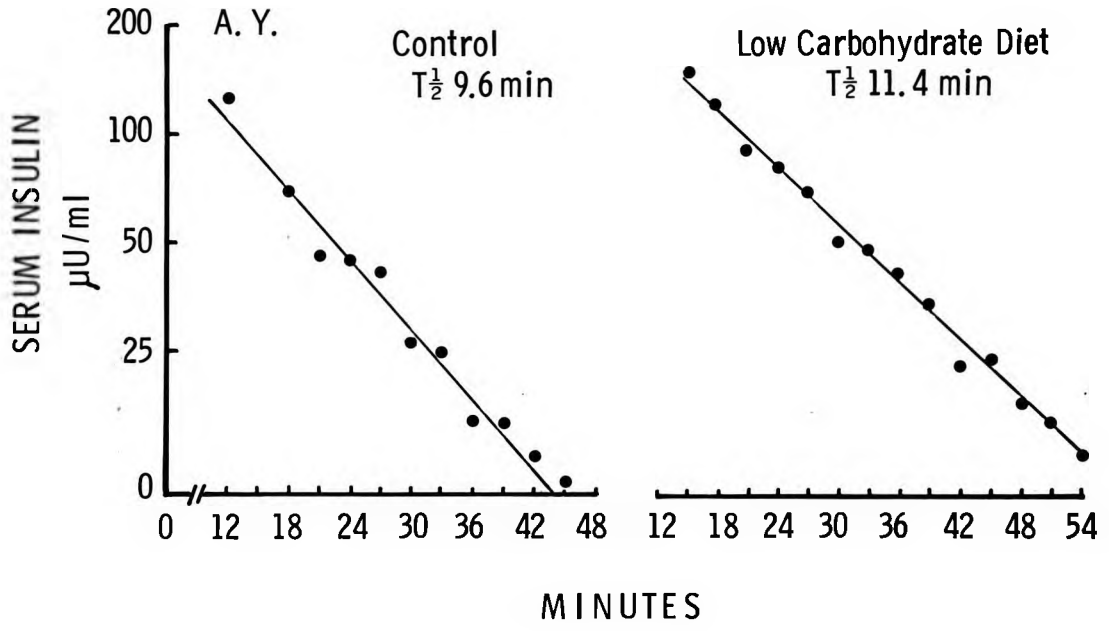

FIG. 23 Influence of carbohydrate restriction on the half-life of exogenous insulin in a normal subject (A.Y.)

the rate at which glucose is required to enter the systemic circulation in order to reproduce the GTT data (Fig. 15). Under control conditions, glucose entry was markedly increased during the periods 0–15 and 15–30 min, but thereafter was strikingly reduced throughout the remainder of the test. In contrast, after carbohydrate restriction glucose continued to reach the general circulation at markedly elevated rates for a full hour after glucose loading. The increments in the amount of glucose reaching the systemic circulation during the entire GTT before and after carbohydrate restriction were 50.7 and 65.3 g respectively.

TABLE 29

Disappearance of exogenous insulin from serum*

	Basal insulin	$T_{\frac{1}{2}}$	$T_{\frac{1}{2}}$ minus basal insulin concentration
	μU/ml	min	min
Control			
M.B.	6	10.5	9.1
A.Y.	14	15.3	9.6
C.H.	14	11.6	9.5
Low carbohydrate diet			
M.B.	6	10.0	8.6
A.Y.	5	11.6	11.4
C.H.	5	10.0	9.0

* for raw data see Appendix , Table 10

TABLE 30

Suggested " balance sheet" for the disposition of 100 g glucose following oral administration before and after carbohydrate restriction in normal subjects

	Before	After
	g	g
Total peripheral glucose uptake	47.9	50.4
Total hepatic glucose conservation	49.2	34.5
Remaining in glucose space after 180 min	2.9	14.1
Loss in urine	0.0	1.0
	100.0	100.0

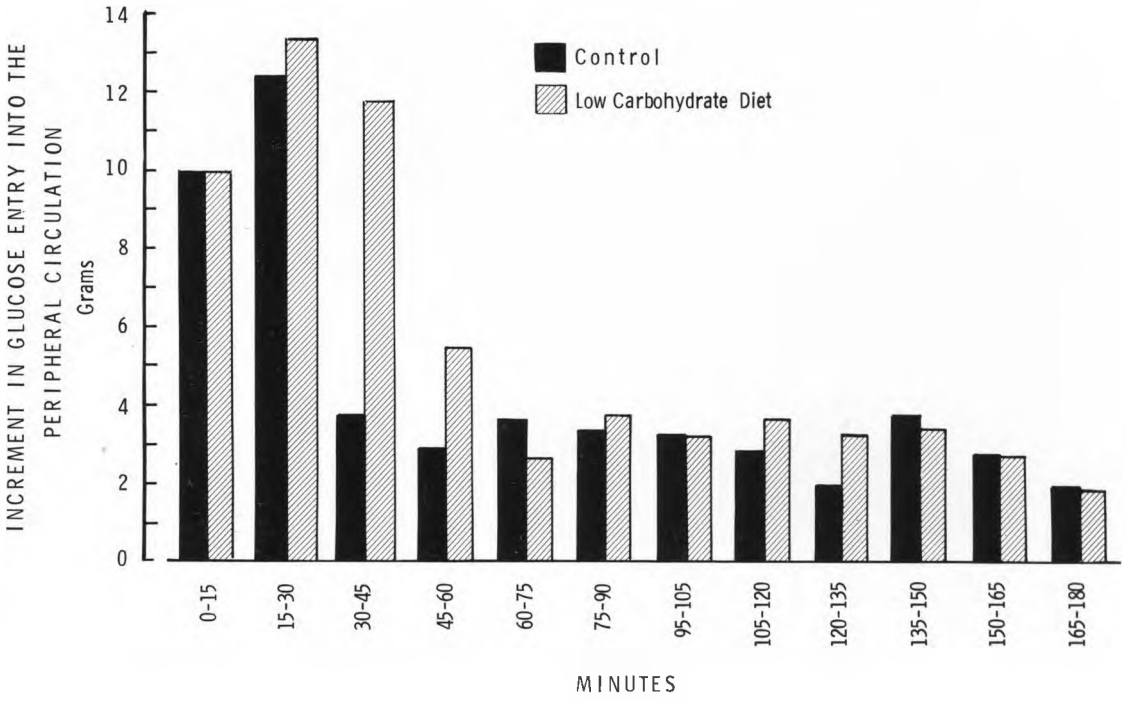

FIG. 24 Influence of carbohydrate restriction on the mean increment in glucose entry into the peripheral circulation following 100 g oral glucose load in 13 normal subjects

DISCUSSION

The present study shows that in normal subjects glucose tolerance was markedly impaired following a low carbohydrate diet, despite the fact that concomitant FGU was not diminished at any phase of the GTT (indeed it was increased between 120-180 min) or during the GTT as a whole (Figs. 15 and 16). These results indicate that decreased peripheral glucose utilisation is not the cause of dietary diabetes in man and suggest rather that impaired glucose uptake by "non-peripheral" tissues must constitute the major underlying disturbance.

No similar study has been reported with which the present findings may be compared, but it has been concluded on the basis of animal experiments that glucose utilisation by skeletal muscle is reduced following carbohydrate deprivation (Randle et al., 1966; Schonfeld and Kipnis, 1968a,b). This disagreement with the present observations is readily explicable from two major standpoints. Firstly, the experimental conditions of the incubation flask are not comparable with the situation existing in vivo during a GTT so that the validity of extrapolating from one situation to the other is questionable. Secondly, since in vitro glycolysis alone was studied while glycogenesis was ignored (Randle et al., 1966; Schonfeld and Kipnis, 1968a,b), these conclusions are based on the assumption that in skeletal muscle glucose utilisation by glycolysis is inevitably the major and glycogenesis the minor determinant of total cellular glucose uptake. While the latter may be so under normal dietary conditions (Beatty et al., 1966), it is almost certainly not so for the glycogen-depleted muscle of carbohydrate-deprived subjects (Hultman and Bergstrom, 1967; Nilsson and Hultman, 1970a). Indeed, in the latter, it seems likely that because the capacity for glycogenesis is increased (Danforth, 1965; Rafaelson et al., 1965; Stauffacher et al., 1965; Bergstrom and Hultman, 1966; Hultman and Bergstrom, 1967) while glycolysis is inhibited (Randle et al., 1966; Schonfeld and Kipnis, 1968a,b), this pattern may be radically altered and even reversed.

It is well established that in skeletal muscle glycogen depletion, however induced, strikingly enhances the capacity of the depleted tissue for glycogen synthesis from glucose (Danforth, 1965; Rafaelson et al., 1965; Stauffacher et al., 1965; Bergstrom and Hultman, 1966; Hultman, 1967; Hultman and Bergstrom, 1967; Moorthy and Gould, 1969). Thus, provided that adequate supplies of glucose are readily available, glycogen-depleted muscle will characteristically exhibit a marked

rebound in glycogenesis so that after resynthesis the glycogen content of previously depleted tissues may exceed the initial value by 100% or more. This response appears to be mediated by both an activation of the enzyme glycogen synthetase (Danforth, 1965) and an increase in the insulin-sensitivity of the pathway leading to glycogen regeneration from glucose (Rafaelsen et al., 1965; Stauffacher et al., 1965) and in my view, is the metabolic basis for the present findings. I propose that in skeletal muscle carbohydrate deprivation induces a redistribution in intracellular glucose disposal so that after glucose loading the increase in glucose utilisation for glycogenesis would counterbalance the concomitant decrease in glucose disposal by glycolysis and thereby quantitatively preserve total cellular glucose uptake (Fig. 25).

This proposal is consistent with the existence in skeletal muscle of glycogenesis and glycolysis as separate independent metabolic pathways each linked to its own glucose-6-phosphate pool (Antony et al., 1969; Dully et al., 1969) and is strongly supported by the recent demonstration in normal subjects (Nilsson and Hultman, 1970a) that the increment in muscle glycogen levels during glucose infusion is enhanced 3-6 fold by the prior ingestion of a low carbohydrate diet. The current findings are also consistent with evidence for a less specific mode of carbohydrate entry into muscle which is insulin-insensitive and which has some of the characteristics of restricted diffusion (Ross, 1956). Thus Ross (1956) suggested that in muscle hyperglycaemia may cause sufficient entry of glucose by this process to compensate for the diminished glucose transport by the more active insulin-sensitive route (Antony et al., 1969; Dully et al., 1969). Although Ross (1956) referred to diabetic tissue, it would seem equally applicable to the muscle of carbohydrate-deprived nondiabetics (Randle et al., 1966; Bloom and Azar, 1963) and could well explain

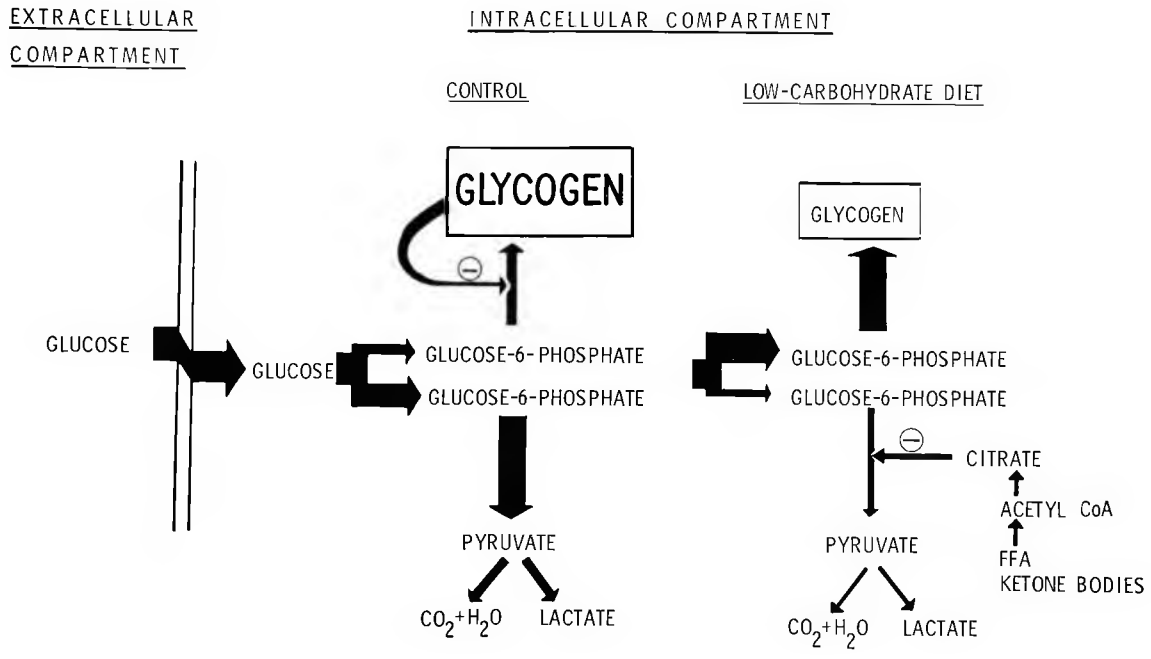

FIG. 25 Suggested patterns of glucose utilisation in skeletal muscle in normally-fed and carbohydrate-deprived normal subjects

the preservation of normal levels of FGU in the early phase of the GTT after carbohydrate deprivation despite the delay in insulin secretion (Fig. 15). It is noteworthy, in passing, that the increased emphasis in glycogen-depleted muscle on the conversion of glucose to glycogen rather than pyruvate (Randle et al., 1963) would lead to glycogen repletion as rapidly as possible, a factor clearly of vital importance in view of the dependence of skeletal muscle on adequate glycogen stores for optimum physical performance (Bergstrom et al., 1967; Hultman, 1967).

The foregoing explanation, though referring solely to skeletal muscle, seems reasonable since both in the forearm and in the periphery of the body as a whole glucose uptake is accounted for mainly by muscle (see Chapter 5). Nevertheless, with the present techniques,

it is not possible to distinguish glucose uptake in adipose tissue from that in muscle and it is conveivable therefore that after the low carbohydrate diet, a diminution in adipose tissue glucose utilisation might have been obscured by the concomitant muscle response; whether or not this is so can ot be confirmed until more detailed observations are made using both deep and superficial venous catheterisation.

The results of the insulin infusion studies (Fig. 18) are consistent with the overall GTT pattern (Table 24) in suggesting that peripheral insulin resistance is increased after carbonydrate restriction. In terms of absolute levels of FGU, however, the infusion studies contrast strikingly with the GTT data (Fig. 15), since during the infusions, FGU was markedly reduced following carbohydrate restriction. Although identical insulin levels were not achieved in each pair of experiments (Fig. 18), the differences were proportionately small and unlikely to account for the reductions in FGU. These results are in agreement with those of Zierler and Rabinowitz (1963) and emphasise the necessity for interpreting experimental findirgs only in terms of the techniques by which they are obtained. The stimulus to tissue glucose utilisation provided by the insulin infusion is not comparable with that resulting during a GTT; thus in the former basal arterial glucose levels are maintained throughout while in the more physiological situation of the latter the tissues are exposed to prolonged elevations in the concentrations of both glucose and insulin. Because the influence on tissue glucose uptake of hyperglycaemia in its own right cannot be ignored (Lundsgaard et al., 1939; Ross, 1956; Park et al., 1959; Randle, 1963), only the GTT data may be regarded as referable to the phenomenon of dietary diabetes. I suggest that after the low carbohydrate diet, it is primarily the hyperglycaemia which promotes glucose uptake and glycogen repletion in

muscle during the GTTs and which, by virtue of its absence, prevents a similar response during the infusion experiments. This role of carbohydrate availability as the principal factor limiting glycogenesis from glucose is supported by recent studies in man (Hultman and Bergstrom, 1967; Nilsson and Hultman, 1970). Thus Hultman and Bergstrom (1967) showed that the ingestion of a carbohydrate-free diet permits only a slow incomplete resynthesis of muscle glycogen after exercise, but that the rebound phenomenon was immediately demonstrable following the administration of a high carbohydrate diet.

Since in the present study dietary diabetes cannot be accounted for by decreased peripheral glucose utilisation, it must be questioned which mechanism is primarily responsible for the impairment in glucose tolerance. Although direct evidence is lacking, the data allow certain deductions to be made. Fig. 24 shows that under control conditions the increment in glucose reaching the peripheral circulation was substantial during the periods 0-15 and 15-30 min but was strikingly reduced thereafter for the remainder of the test. As noted in Chapter 5, ingested glucose can reach the peripheral circulation only through the hepatic veins and because after absorption the liver is the first organ to be perfused by the glucose, any increase in hepatic glucose uptake will simultaneously reduce the amount of glucose available for subsequent entry into the systemic vascular bed. It has been suggested (Chapter 5) that this reduction in glucose entry observed after 30 min is a reflection of increasing hepatic uptake of the absorbed glucose load. After a low carbohydrate diet, this effect was delayed (Fig. 24) so that for a further min substantial quantities of glucose continued to bypass the liver and enter the systemic circulation, thus further elevating arterial glucose levels and resulting in the impairment in glucose tolerance

observed. In association with this delay in hepatic glucose uptake, there was a reduction by some 30% in overall hepatic glucose conservation during the GTT (Table 30); in the presence of undiminished peripheral glucose uptake, therefore, it may be concluded that it is these changes in hepatic glucose metabolism which are the primary causes of dietary diabetes.

The studies of several investigators support this conclusion. Thus, it has been established that the rate-limiting step in hepatic glucose utilisation is the rate of glucose phosphorylation (Cahill et al., 1959), that the activity of the enzyme catalysing glucose phosphorylation in liver, viz. glucokinase, is reduced by starvation (Sharma et al., 1964; Katzen, 1967) or a low-carbohydrate diet (Borreback et al., 1970), that on refeeding several hours are required before glucokinase activity increases following carbohydrate restriction (Salas et al., 1965; Borreback et al., 1970), that following starvation a direct relationship exists between hepatic glucokinase activity and glucose tolerance (Hornichter and Brown, 1969), that the alternative enzyme catalysing glucose phosphorylation in liver, viz. pyrophosphate glucose phosphotransferase, is probably effective only at markedly elevated glucose concentrations (McCraw, 1968), and that in liver, unlike muscle, glucose utilisation must proceed initially by phosphorylation into a single glucose-6-phosphate pool linked to both glycogenesis and glycolysis since alternative pathways for glucose incorporation into glycogen do not exist (Antony et al., 1969). In the perfused rat liver, McCraw (1968) has confirmed that indeed hepatic glucose utilisation is reduced by starvation, while Landau et al. (1961) demonstrated in dogs that the sensitivity of the liver to changes in blood glucose concentrations was considerably decreased by the prior restriction of dietary carbohydrate. These workers (Landau et al., 1961) found that during

glucose infusions in animals receiving a high carbohydrate diet, blood glucose levels increased very little, reaching only 100-130 mg/100 ml yet despite this net hepatic storage of glucose occurred; in contrast in carbohydrate-deprived animals, there was a significantly greater rise in glucose concentrations and only at hyperglycaemic levels (150-200 mg/100 ml) was net hepatic glucose uptake observed.

Whether the delayed hepatic uptake of glucose during the GTTs is entirely attributable to the concomitant delay in insulin secretion (Tzagournis and Skillman, 1970) found in the majority of subjects (Table 23) or primarily to an hepatic unresponsiveness conditioned by the fall on glucokinase activity or both remains unclear, but data from two subjects (G.T. and G.M.) suggests that in fact low glucokinase activity was the governing factor in this response. Thus Fig. 17 (see Appendix, Table 7) illustrates the development of marked glucose intolerance in one of these volunteers despite the normally prompt rise and subsequent greater elevation in serum insulin concentrations. These changes in peripheral insulin levels suggest that concomitant insulin secretion into the portal circulation was, similarly, normally prompt (Blackard and Nelson, 1970), since neither insulin half-life (Table 29) nor hepatic insulin uptake (Marshall et al., 1970) are reduced by carbohydrate restriction. Accordingly, these findings (Fig. 17) are consistent with the failure of the liver to respond rapidly to a glucose load despite undiminished insulin secretion by the islets. This conclusion is not invalidated by the finding that, following starvation, the ratio "Big" insulin/total immunoassayable insulin is increased (Gordon and Roth, 1969), since proinsulin (Melani et al., 1970) which is probably the same compound as "Big" insulin appears to be biologically as effective as mature insulin in the liver (Rees and Madison, 1969), at least as regards the suppression of hepatic glucose output. The data on

insulin half-life (Table 29) are in accordance with the findings of Samols and Marks (1966) and Ørskov and Christensen (1969); others (Stimmler et al., 1969) have reported a shorter half-life for insulin.

The lactate data (Fig. 19) support the proposal that hepatic glucose uptake is delayed after carbohydrate restriction. Fig. 19 shows that after a normal diet glucose loading resulted in an immediate and substantial rise in lactate levels but not, in contrast, after carbohydrate restriction. Comparable changes in the shape of lactate response curves following starvation have been reported previously (Sussman, 1966; Jackson et al., 1971), but their interpretation has remained uncertain hitherto. It has been concluded, however, that since the early rise in plasma lactate concentrations during the control GTT takes place in association with continuing peripheral lactate uptake, this rise must be the result of a prompt increase in hepatic lactate release, i.e. the consequence of enhanced hepatic glucose uptake, its conversion to lactate by glycolysis with the subsequent release of lactate into the circulation (see Chapter 5). Accordingly, the ablation of this early rise in lactate concentrations after the low carbohydrate diet (Fig. 19) suggests that at this time the increase in hepatic glucose utilisation was markedly attenuated and considerably delayed. These conclusions are strongly supported by recent studies of lactate production in the perfused rat liver (Craig, 1966; Woods and Krebs, 1971) and are in keeping with the fall in the activity of the key glycolytic enzymes in liver following carbohydrate restriction (Weber et al., 1966).

Interestingly, other authors (Martinsson et al., 1963; Sunzel, 1963; Nilsson and Hultman, 1970a) have found that following carbohydrate restriction, the increment in liver glycogen content after glucose administration was either unchanged or enhanced. The existence of a delay in hepatic glucose uptake is not at variance with

these studies since after carbohydrate restriction, the regeneration of liver glycogen, in contrast to muscle glycogen, probably takes place in the first instance from lactate and only subsequently from glucose (Samols and Holdsworth, 1968; Nilsson and Hultman, 1970b; Zaragoza and Felber, 1970). This is consistent with the initial fall in lactate levels after glucose loading (Fig. 19) which must be regarded as indicative of early hepatic lactate uptake, since only enhanced lactate utilisation by "nonperipheral" tissues, viz. the liver, could reduce systemic lactate concentrations in the presence of continuing peripheral lactate release. Comparable patterns in lactate balance have been reported in the perfused rat liver after starvation (Craig, 1966).

Teleologically the present data suggest that the adaptive decrease in hepatic glucokinase activity consequent on carbohydrate restriction (Borreback et al., 1970) subserves the important function of ensuring, on the re-introduction of dietary carbohydrate, the rapid and optimal restoration of peripheral glycogen stores. Thus, by virtue of the prevailing block to glucose phosphorylation glucose uptake at hepatic level is prevented during the initial phase of refeeding. As a result, newly absorbed glucose is immediately diverted to the peripheral circulation and there retained in high concentration for preferential uptake by tissues which, unlike the liver, cannot reverse glycolysis and which, therefore, cannot utilise substrates other than glucose for glycogenesis. The dependence of skeletal muscle on adequate glycogen stores for optimum physical performance has been referred to above.

Some workers have reported an increased rate of glucose absorption from the gut after carbohydrate restriction (Kershaw et al., 1960; Dowling, 1967; Esposito, 1967) and it becomes theoretically possible, therefore, that an increased rate of glucose absorption is a

major cause of dietary or starvation diabetes. The findings of other authors, however, suggest that changes in the rate of glucose absorption are unlikely to influence significantly the form of the glucose tolerance curve (Holdsworth, 1969) and indeed Verdy (1970) found no change in xylose absorption after starvation in obese females. It seems improbable, therefore, that an increase in glucose absorption alone could have played anything more than a marginal role in producing the disturbance observed (Fig. 15).

Basally, lactate release by the forearm was significantly increased by carbohydrate restriction although glucose uptake remained unchanged. Zierler and Rabinowitz (1963) have similarly reported an increase in basal lactate production by forearm muscle after starvation and suggested that this increased lactate production could account for all the concomitant glucose uptake. It is hardly likely, however, that muscle glycolysis could be enhanced by starvation (Randle et al., 1966); Schonfeld and Kipnis, 1968a) and, in keeping with the conclusions of Kreisberg et al. (1970), it seems far more probable that under basal conditions the majority of the lactate release by the forearm was derived from precursors other than glucose. Accordingly, the present observations suggest that the cyclic process of glucose breakdown in peripheral tissues with the formation of lactate which is transported to the liver for resynthesis into glucose (Cori Cycle) may be strikingly modified by a low carbohydrate diet. Thus it would appear that basally the glucose released by the liver is converted in muscle not to lactate but to glycogen, while the lactate released by muscle and which is destined for hepatic uptake, is derived not from glucose but from amino acids.

The changes in acetoacetate and β-hydroxybuturate concentrations after the low carbohydrate diet differed strikingly both with regard to the shape of the respective response curves after glucose loading

and in terms of the trends in metabolic balance observed across the forearm. Glucose loading induced an initial rise in acetoacetate but an immediate fall in β-hydroxybutyrate concentrations; furthermore, acetoacetate was taken up by the forearm while, conversely, β-hydroxybutyrate was released (Fig. 20). In order to explain the rise in acetoacetate levels in the face of continuing peripheral uptake, a transient increase in non-peripheral, i.e. hepatic, acetoacetate release must be postulated, while the fall in β-hydroxybutyrate concentrations, despite increasing peripheral release of this compound can be accounted for only by an immediate fall in hepatic production. Thus, with regard to ketone bodies as well as lactate, the shapes of the respective response curves appear to be determined at hepatic rather than peripheral level, and in view of the role of the liver as the major site of ketogenesis (Bressler, 1963) this is not surprising. The biochemical basis of these findings, however, remains obscure, but Fig. 21 suggests that whatever the mechanisms involved, there does not appear to be a simple quantitative conversion of acetoacetate to β-hydroxybutyrate in the periphery. Thus, although forearm acetoacetate uptake decreases progressively after glucose loading, β-hydroxybutyrate release rose initially and exceeded that which could be accounted for by concomitant acetoacetate uptake.

The findings are supported by many previous reports. Thus a dissociation between the acetoacetate and β-hydroxybutyrate response curves similar to that shown in Fig. 20, has been demonstrated previously in man (Gammeltoft, 1951; Rooth et al., 1969). Furthermore, several authors (Gammeltoft, 1951; McCann, 1957; Beatty et al., 1960; Williamson and Krebs, 1961; Bressler, 1963; Beatty et al., 1964; Söling et al., 1965; Hagenfeldt and Wahren, 1968; Owen and Reichard, 1971) have suggested that muscle may take up acetoacetate and release β-hydroxybutyrate and that of the acetoacetate taken up, only a minor

proportion is converted to β-hydroxybutyrate. More recently, Bieberdorf et al. (1970) have confirmed that indeed acetoacetate and β-hydroxybutyrate do not exist as parts of a single rapidly mixing pool. Whether the observed changes in acetoacetate and β-hydroxybutyrate concentrations during the GTTs (Fig. 20) are due to insulin action directly, or to enhanced glucose utilisation, or both, remains uncertain and clearly, this field requires further detailed research.

SUMMARY

FGU and lactate balance across the forearm were determined in normal subjects during 100 g GTTs before and after a low carbohydrate diet; in addition, β-hydroxybutyrate and acetoacetate responses were measured following carbohydrate restriction.

After a low carbohydrate diet, glucose tolerance was significantly impaired (dietary diabetes) and the rise in serum insulin levels was frequently but not invariably delayed. FGU, however, was not significantly decreased at any phase of the GTT or during the GTT as a whole.

On a normal diet, lactate levels rose immediately after glucose loading, reaching peak concentrations at 60 min and falling progressively thereafter; this rise was associated with lactate uptake by the forearm and the subsequent fall in plasma concentrations with lactate release. In contrast following carbohydrate restriction, lactate concentrations were initially reduced by glucose administration, this reduction taking place despite continuing lactate release from the forearm. These results suggest that after glucose loading, the shape of the lactate response curve is not determined primarily by concomitant changes in peripheral lactate balance.

Marked elevations in basal FFA, β-hydroxybutyrate and acetoacetate levels accompanied carbohydrate restriction. FFA and β-hydroxybutyrate concentrations were immediately decreased, but acetoacetate levels initially increased by glucose loading, the latter declining only during the second hour of the GTT; throughout the test, acetoacetate was taken up by the forearm while β-hydroxybutyrate was released. Basal FFA and β-hydroxybutyrate concentrations were not correlated with the degree of glucose tolerance induced in the volunteers.

The results show that the impairment of glucose tolerance following carbohydrate restriction is not the result of decreased peripheral glucose utilisation. It is suggested that the major cause of dietary diabetes is a delay in the hepatic uptake of the glucose load and that this is primarily the result of low hepatic glucokinase activity rather than delayed insulin secretion.

CHAPTER 7

THE RELATIONSHIP BETWEEN THE BASAL GLUCOSE CONCENTRATION, GLUCOSE TOLERANCE AND FOREARM GLUCOSE UPTAKE IN MATURITY-ONSET DIABETES

INTRODUCTION

It is widely acknowledged that in diabetics a low carbohydrate diet results in a fall in the basal glucose concentration and a lowering of the glucose tolerance curve - effects to which the majority of authors (Beck et al., 1964; Yalow et al., 1965; Schless and Duncan, 1966; Jackson et al., 1968; Jackson et al., 1971) refer as an "improvement in glucose tolerance". There is reason to suspect, however, that in the past this interpretation has been frequently incorrect. Thus, the level of the basal glucose concentration profoundly influences the glucose concentrations which result after glucose loading and it is conceivable, therefore, that the lowering of the glucose tolerance curve by carbohydrate restriction is not necessarily indicative of "improved glucose tolerance" but merely an effect of the fall in the basal glucose concentration.

Changes in basal glucose levels and glucose tolerance are determined by largely unrelated processes and accordingly should be treated as separate entities, the former depending principally on the rate of gluconeogenesis (Cahill, 1971), while the latter refers primarily to those processes which determine the capacity for glucose disposal and the restoration of basal glucose concentrations after glucose ingestion. Clearly, therefore, glucose tolerance is best expressed as the incremental area under the arterial glucose tolerance curve rather than the total area since the former but not the latter eliminates the influence on the curve due solely to changes in the basal concentration.

The possibility that carbohydrate restriction, while reducing fasting hyperglycaemia and lowering the glucose curve, may further impair glucose tolerance in some diabetics has been noted by Jackson et al. (1971); these workers, however, confined their observations

to the measurement of concentrations alone and thus could not localise the site(s) of such impairment. Although Butterfield and Whichelow (1968) did study peripheral glucose uptake during GTTs in diabetics after dietary restriction, they failed to interpret their findings in terms of glucose tolerance in the body as a while. Accordingly the present investigation documents FGU during 100 g GTTs in diabetics before and after carbohydrate restriction in order to analyse the relationship between the change in glucose tolerance (defined as the incremental area under the glucose tolerance curve) and the concomitant changes in the basal glucose concentration and peripheral glucose uptake.

SUBJECTS AND METHODS

Six non-obese, non-insulin requiring diabetic males, none of whom suffered from hepatic or renal insufficiency or other endocrine disease, were studied (Table 31); all subjects gave informed consent. Five subjects had fasting hyperglycaemia and a low insulin response to glucose loading and will be reported together; the sixth subject (J.W.) is described separately. In each subject, a GTT was performed after 10 days on a normal carbohydrate intake and again, with the exception of J.W., after seven days on a low carbohydrate diet, each individual acting as his own control. All subjects remained fully ambulant throughout. Lactate, acetoacetate and β-hydroxybutyrate were estimated on blood precipitated immediately on withdrawal in ice-cold 0.6M perchloric acid. All therapy was withheld throughout the period of study, beginning at least 10 days prior to the first (control) GTT and being reinstituted only after completion of the second GTT.

TABLE 31

Clinical data

Subject	Age	Height	Weight	Total weight loss	Known duration of diabetes	Family history of diabetes	Previous therapy
	yr	cm	kg	kg	yr		
V.P.	62	174	70.7	3.5	8	0	Chlorpropamide
T.R.	47	183	77.5	3.	< 1	+	Nil
P.R.	60	172	70.0	4.0	14	0	Chlorpropamide
K.P.	30	185	84.5	2.9	< 1	+	Carbohydrate restriction
J.L.	55	163	69.8	2.4	< 1	+	Clofibrate
J.W.	41	180	81.0	-	< 1	0	Nil

RESULTS

Fig. 26 (Tables 32 and 33) shows that on a normal diet basal glucose concentrations were elevated, glucose tolerance was grossly impaired and the insulin response markedly reduced (Buchanan and McKiddie, 1967; Perley and Kipnis, 1967; Reaven and Miller, 1968; see Chapter 6, Table 16). Mean FGU increased to reach peak levels 45 min after glucose ingestion, declining thereafter; the rise in FGU (expressed as the incremental area 0-180 min) amounted to 52.8 mg/100 ml forearm. After a low carbohydrate diet, basal glucose concentrations were strikingly reduced ($p < .01$) but the overall increment in AV glucose concentrations after glucose loading (expressed as the incremental area 0-180 min) remained unchanged, as did the rise in serum insulin concentrations (Fig. 27; see Appendix, Table 12). The increment in FGU (0-180 min) was increased to 76.5 mg/100 ml forearm but this change was not statistically significant; when considered hour by hour, however, there was a significantly greater increase in both FGU ($p < .05$) and AV glucose concentrations ($p < .02$) between 120-180 min than occurred on a normal diet. Mean ($\pm$ SEM) blood flow during the GTTs was significantly less ($p < .001$) after carbohydrate restriction, the levels being respectively 3.5 $\pm$ 0.2 and 2.6 $\pm$ 0.1 ml/100 ml forearm/min before and after the low carbohydrate diet.

On a normal diet, lactate concentrations rose in response to glucose loading (Fig. 28; Table 34) but AV and MV levels were not significantly different. In contrast, during the GTT following carbohydrate restriction, lactate levels tended to fall slightly; MV concentrations were significantly higher than corresponding AV levels at both 30 ($p < .05$) and 60 ($p < .05$) min. The basal AV lactate concentrations after carbohydrate restriction were lower but not significantly so.

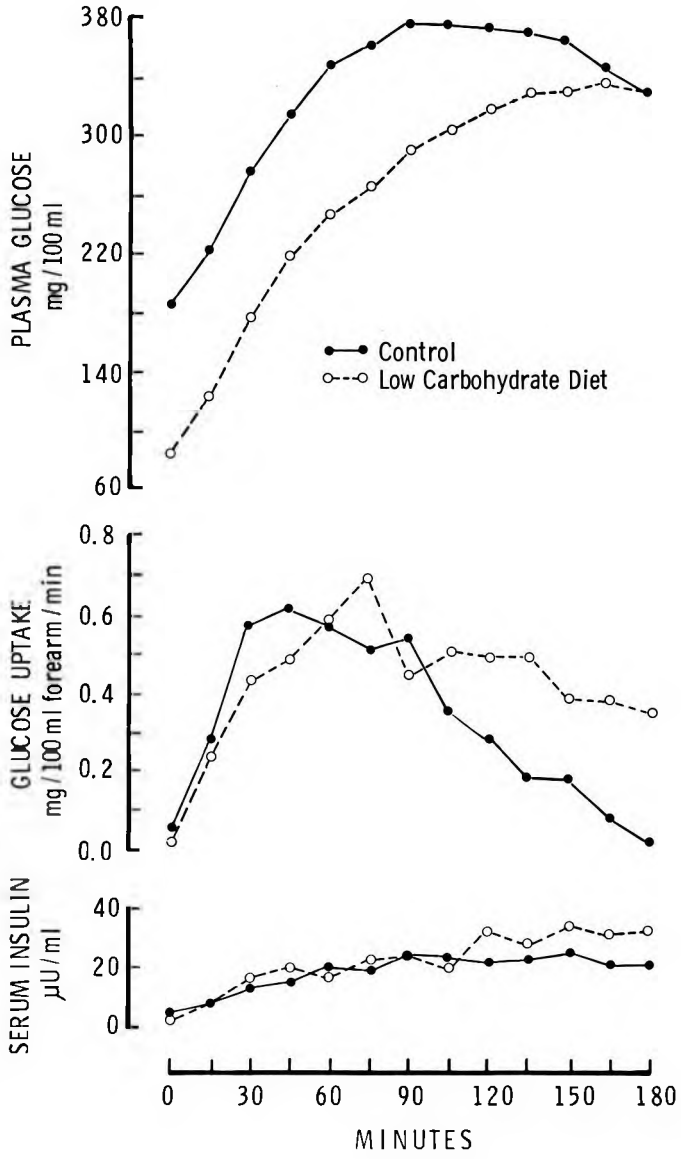

FIG. 26 Influence of carbohydrate restriction on the mean response following 100 g oral glucose load in five maturity-onset diabetics. The glucose curves illustrate the concentrations in arterialised venous plasma

TABLE 32

Mean response following 100 g oral glucose load after normal diet in five diabetics

	Minutes						
	0	15	30	45	60	75	90
Glucose AV* mg/100 ml	183 ± 23**	220 ± 19	273 ± 18	313 ± 21	345 ± 25	357 ± 25	373 ± 28
MV§	179 ± 24	197 ± 21	233 ± 17	275 ± 19	315 ± 23	332 ± 25	343 ± 26
Glucose uptake mg/100 ml forearm/min	.066 ± .030	.289 ± .042	.573 ± .071	.618 ± .088	.571 ± .103	.515 ± .089	.544 ± .112
Insulin μU/ml	7 ± 1	11 ± 2	16 ± 4	18 ± 5	22 ± 6	23 ± 7	26 ± 7
FFA μmol/l	576 ± 94	–	571 ± 92	–	424 ± 58	–	374 ± 83
Growth hormone ng/ml	6.4 ± 3.1	–	2.7 ± 0.9	–	2.0 ± 0.3	–	6.4 ± 5.0
Blood flow ml/100 ml forearm/min	2.5 ± 0.2	2.2 ± 0.3	2.3 ± 0.3	2.7 ± 0.2	3.2 ± 0.5	3.7 ± 0.8	3.3 ± 0.4

* Arterialised venous
** Standard error of mean
§ Mixed venous

TABLE 32 Cont.

Mean response following 100 g oral glucose load after normal diet in five diabetics

	Minutes					
	105	120	135	150	165	180
Glucose AV* mg/100 ml	373 ± 27	369 ± 29	366 ± 31	361 ± 29	344 ± 26	328 ± 23
MV ς	356 ± 25	356 ± 28	358 ± 32	353 ± 30	343 ± 28	328 ± 26
Glucose uptake mg/100 ml forearm/min	.364 ± .071	.297 ± .076	.199 ± .089	.197 ± .093	.090 ± .090	.037 ± .037
Insulin µU/ml	26 ± 7	24 ± 7	25 ± 7	28 ± 8	24 ± 6	24 ± 6
FFA µmol/l	–	291 ± 47	–	239 ± 43	–	278 ± 22
Growth hormone ng/ml	–	3.7 ± 1.7	–	6.2 ± 2.1	–	6.2 ± 2.6
Blood flow ml/100 ml forearm/min	3.6 ± 0.4	4.0 ± 0.6	4.0 ± 0.8	4.7 ± 0.6	4.6 ± 0.7	4.4 ± 0.7

* Arterialised venous
** Standard error of mean
ς Mixed venous

TABLE 33

Mean response following 100 g oral glucose load after carbohydrate restriction in five diabetics

		Minutes						
		0	15	30	45	60	75	90
Glucose ng/100 nl	AV*	85 ± 7**	122 ± 8	175 ± 11	216 ± 12	244 ± 11	269 ± 14	288 ± 14
	p §	<.02	<.01	<.02	<.02	<.02	<.02	<.02
	MV ‡	83 ± 8	102 ± 7	139 ± 8	182 ± 12	205 ± 8	228 ± 10	261 ± 14
	p	<.02	<.02	<.02	<.05	<.02	<.05	<.02
Glucose uptake ng/100 nl forearm/min		.028 ± .009	.240 ± .085	.434 ± .108	.491 ± .117	.589 ± .159	.695 ± .194	.455 ± .105
	p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Insulin uU/ml		5 ± 1	11 ± 2	19 ± 4	22 ± 7	19 ± 3	25 ± 5	26 ± 4
	p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
FFA umol/l		989 ± 107	–	880 ± 102	–	685 ± 103	–	571 ± 90
	p	N.S.		N.S.		N.S.		N.S.
Growth hormone ng/ml		17.4 ± 7.5	–	10.7 ± 4.1	–	3.8 ± 1.2	–	3.5 ± 1.2
	p	N.S.		N.S.		N.S.		N.S.
Blood flow ml/100 ml forearm/min		2.7 ± 0.1	1.8 ± 0.2	1.9 ± 0.2	2.3 ± 0.4	2.4 ± 0.5	2.8 ± 0.5	2.8 ± 0.4
	p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Arterialised venous

** Standard error of mean

§ Significance of difference between means before and after carbohydrate restriction

‡ Mixed venous

TABLE 33 Cont.

Mean response following 100 g oral glucose load after carbohydrate restriction in five diabetics

		Minutes					
		105	120	135	150	165	180
Glucose mg/100 ml	AV*	303 ± 19**	316 ± 17	326 ± 18	328 ± 15	332 ± 15	329 ± 16
	p§	< .05	N.S.	N.S.	N.S.	N.S.	N.S.
	MV‡	273 ± 15	289 ± 14	298 ± 15	307 ± 16	314 ± 17	310 ± 17
	p	< .02	< .05	N.S.	N.S.	N.S.	N.S.
Glucose uptake mg/100 ml forearm/min		.506 ± .119	.499 ± .158	.499 ± .132	.395 ± .125	.385 ± .159	.359 ± .096
	p	N.S.	N.S.	< .05	N.S.	< .02	< .01
Insulin uU/ml		22 ± 2	33 ± 6	29 ± 2	35 ± 7	33 ± 4	34 ± 5
	p	N.S.	N.S.	N.S.	N.S.	< .05	< .01
FFA umol/l		–	579 ± 80	–	597 ± 83	–	598 ± 88
	p		N.S.		N.S.		< .05
Growth hormone ng/ml		–	2.6 ± 0.9	–	3.1 ± 0.6	–	3.4 ± 0.6
	p		N.S.		N.S.		N.S.
Blood flow ml/100 ml forearm/min		2.8 ± 0.4	2.9 ± 0.4	2.8 ± 0.4	3.0 ± 0.5	3.0 ± 0.5	3.2 ± 0.4
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Arterialised venous
** Standard error of mean
§ Significance of difference between means before and after carbohydrate restriction
‡ Mixed venous

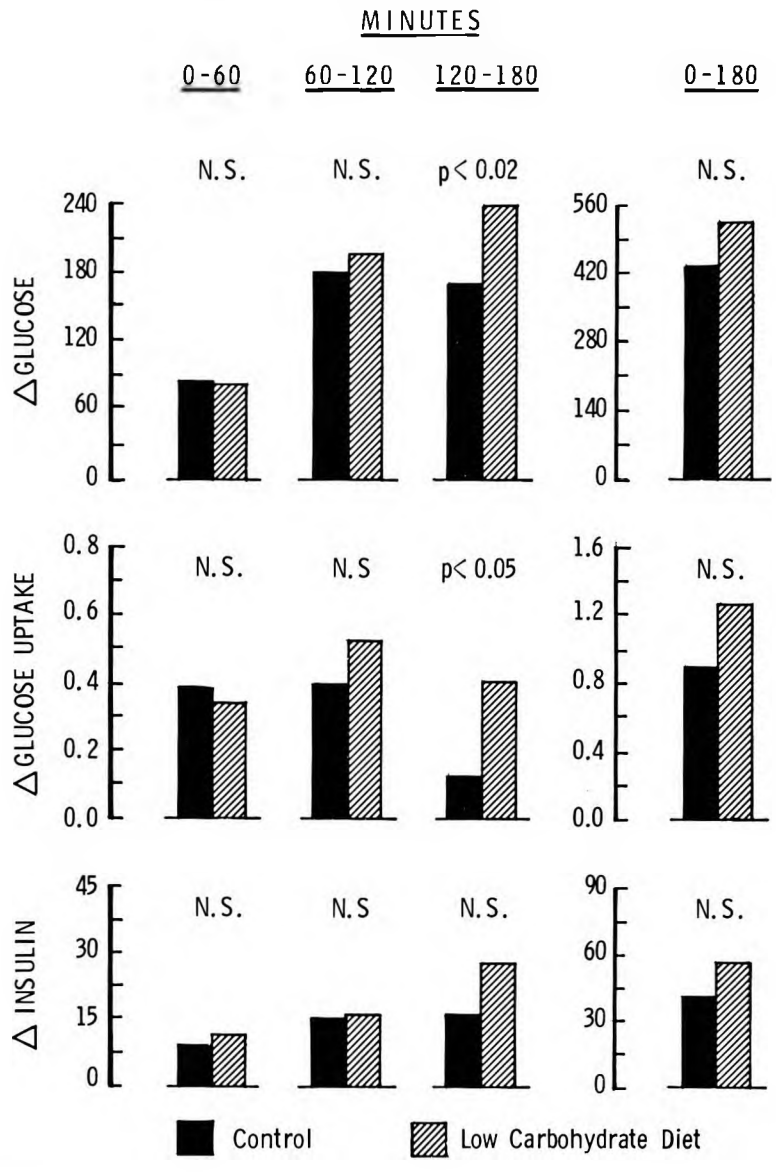

FIG. 27 Influence of carbohydrate restriction on the mean response following 100 g oral glucose load expressed as the incremental area under the curve (Δ) in five maturity-onset diabetics

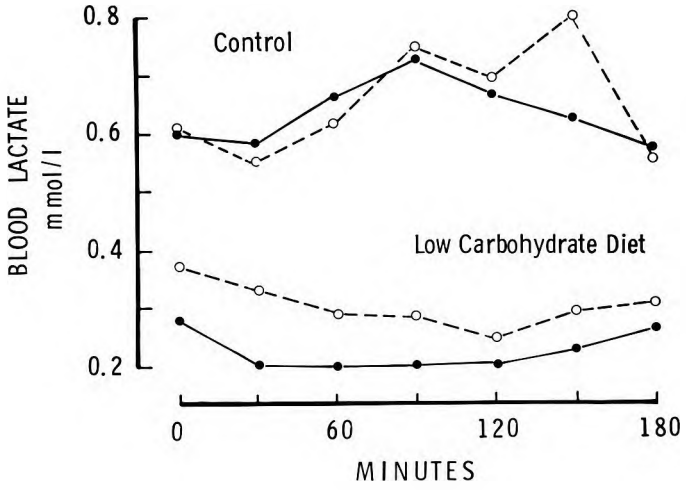

FIG. 28 Influence of carbohydrate restriction on the mean changes in lactate concentrations in arterialised venous (●—●) and mixed venous (o--o) blood following 100 g oral glucose load in four maturity-onset diabetics

Basal FFA concentrations rose in each subject after carbohydrate restriction and, as with the AV lactate levels, the failure of this change to achieve statistical significance is probably a consequence of having only five subjects in this study. FFA concentrations were reduced by glucose loading both before and after carbohydrate restriction. Serum growth hormone responses were not influenced by carbohydrate restriction and the high basal value is due to bias by the findings in one individual (T.R.).

After a normal carbohydrate intake, neither acetoacetate nor β-hydroxybutyrate were detectable in blood but basally, substantial ketonaemia was observed after the low carbohydrate diet (Fig. 29; Table 35). A clear dissociation was observed between the shapes of the acetoacetate and β-hydroxybutyrate response curves; thus while β-hydroxybutyrate concentrations fell immediately ($p < .05$ at 30 min)

TABLE 34

Mean lactate concentrations (mmol/l) in arterialised venous (AV) and mixed venous (MV) blood following 100 g oral glucose load in four diabetics

	Minutes						
	0	30	60	90	120	150	180
Control							
AV	.60 ± .09*	.58 ± .09	.66 ± .09	.72 ± .06	.66 ± .06	.62 ± .06	.57 ± .07
MV	.61 ± .07	.55 ± .06	.61 ± .06	.74 ± .06	.69 ± .03	.79 ± .14	.55 ± .01
p**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Low carbohydrate diet							
AV	.28 ± .03	.20 ± .03	.20 ± .02	.20 ± .03	.20 ± .03	.22 ± .03	.26 ± .05
MV	.37 ± .06	.33 ± .05	.29 ± .04	.28 ± .05	.25 ± .03	.29 ± .05	.30 ± .05
p	N.S.	<.05	< .05	N.S.	N.S.	N.S.	N.S.

* Standard error of mean

** Significance of difference between means before and after carbohydrate restriction

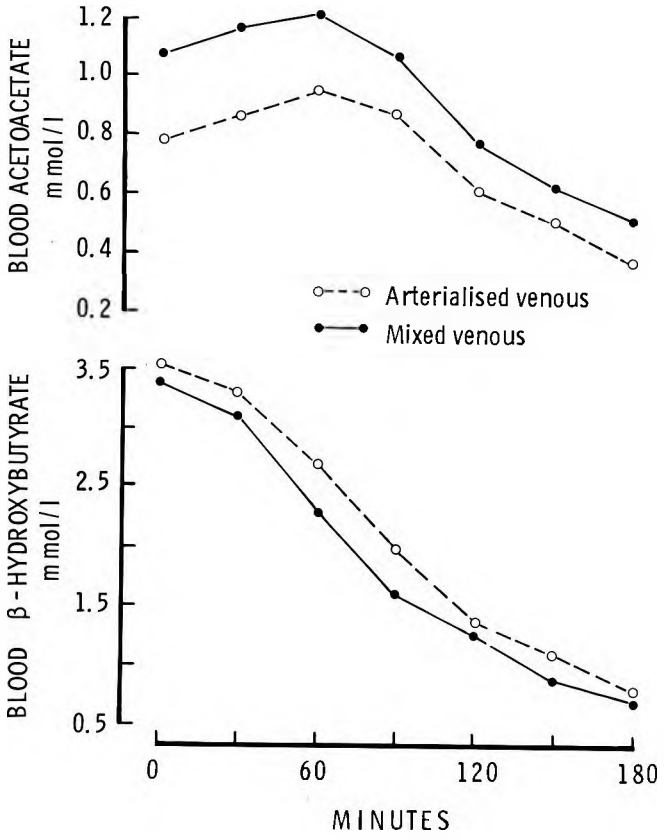

FIG. 29 Mean changes in acetoacetate and β-hydroxybutyrate concentrations following 100 g oral glucose load after carbohydrate restriction in four maturity-onset diabetics

and continued to decline throughout the GTT, acetoacetate levels remained unchanged for as long as 90 min falling only thereafter. Both basally and after glucose loading, acetoacetate was taken up by the forearm while β-hydroxybutyrate was released. Statistically significant AV-MV differences were found for acetoacetate at 0 ($p<.01$), 30 ($p<.01$), 60 ($p<.01$) and 90 ($p<.05$) min and for β-hydroxybutyrate at 30 ($p<.02$), 60 ($p<.02$) and 90 ($p<.02$) min.

Serum potassium concentrations were not influenced by carbohydrate restriction (Table 36).

Figs. 30 - 32 (see Appendix, Tables 13-15) compare the responses

TABLE 35

Mean acetoacetate and β-hydroxybutyrate concentrations (mmol/l) in arterialised venous (AV) and mixed venous (MV) blood following 100 g oral glucose load after carbohydrate restriction in four diabetics

	Minutes						
	C	30	60	90	120	150	180
Acetoacetate							
AV	1.07 ± .08*	1.16 ± .06	1.20 ± .10	1.05 ± .14	0.78 ± .11	0.61 ± .16	0.51 ± .20
MV	0.78 ± .07	0.85 ± .10	0.93 ± .08	0.86 ± .11	0.60 ± .17	0.49 ± .12	0.36 ± .13
p**	<.01	<.01	<.01	<.05	N.S.	N.S.	N.S.
β-hydroxybutyrate							
AV	3.4 ± 0.4	3.1 ± 0.4	2.3 ± 0.3	1.6 ± 0.3	1.3 ± 0.3	0.9 ± 0.3	0.7 ± 0.3
MV	3.5 ± 0.3	3.3 ± 0.4	2.7 ± 0.3	2.0 ± 0.3	1.4 ± 0.3	1.1 ± 0.3	0.8 ± 0.3
p	N.S.	<.02	<.02	<.02	N.S.	N.S.	N.S.

* Standard error of mean

** Significance of difference between means

TABLE 36

Serum potassium (mEq/l) before and after carbohydrate restriction

Subject	Before	After
V.P.	4.0	4.3
T.R.	4.1	4.1
P.R.	4.6	4.3
K.P.	4.6	4.4
J.L.	4.4	4.9

of each of three diabetics with those of age-matched normal male subjects. K.P. (Fig. 30) and T.R. (Fig. 31) had fasting hyperglycaemia and a low insulin response to glucose challenge while J.M. (Fig. 32) had fasting normoglycaemia and a hyperinsulinaemic response to glucose loading. Fig. 30 shows that by 90 min the increments in FGU in K.P. and J.B. were 35.2 and 25.7 mg/100 ml forearm respectively yet the increment in plasma glucose concentrations in K.P. was 162 mg/100 ml compared with only 31 mg/100 ml in J.B. Although in the GTT as a whole the increments in FGU were virtually identical in the two subjects (47.3 and 45.0 mg/100 ml forearm in K.P. and J.B. respectively), the elevation in glucose concentrations remaining at 180 min was 199 mg/100 ml in the diabetic as against 17 mg/100 ml in J.B. and the incremental area under the glucose curve in K.P. was 2.6-fold that in the non-diabetic. Fig. 31 shows a similar pattern of disturbance in T.R., i.e. that while after 120 min the increments in FGU were practically identical in T.R. and T.P. (77.5 and 76.0 mg/100 ml forearm respectively), the corresponding elevations in glucose concentrations

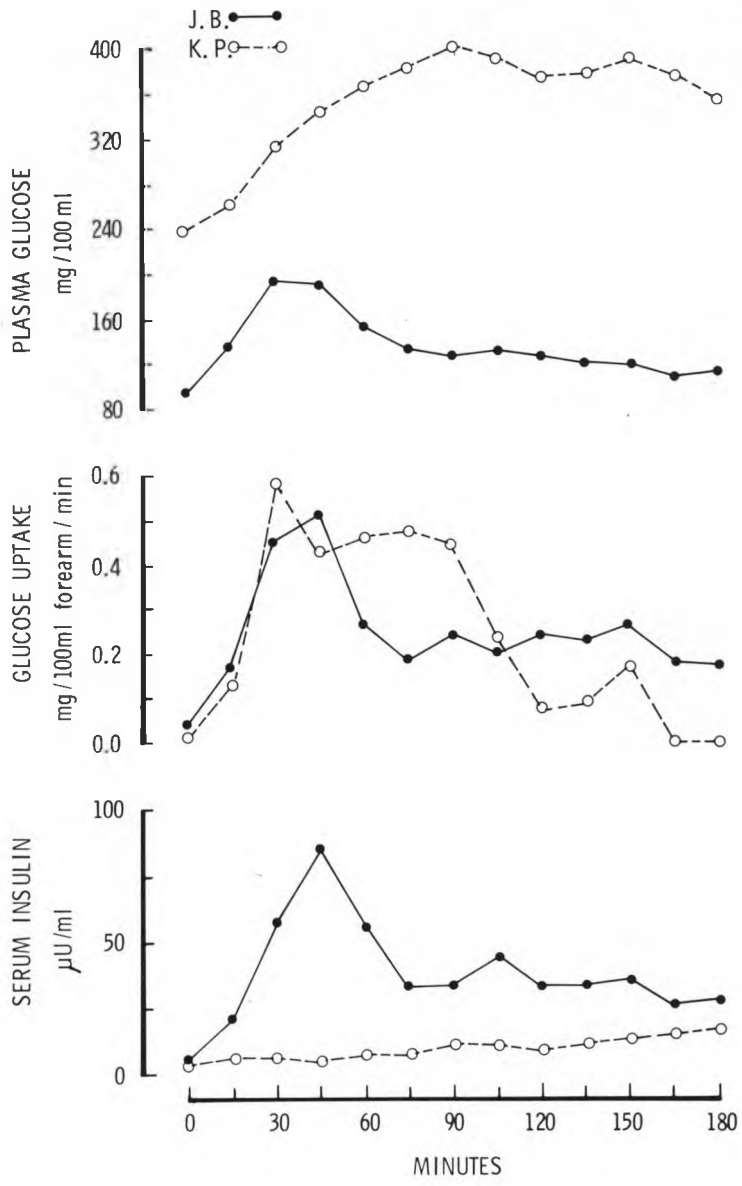

FIG. 31 Comparison of response following 100 g oral glucose load in diabetic K.P. (Table 31) and J.B. - a 30 year old normal male

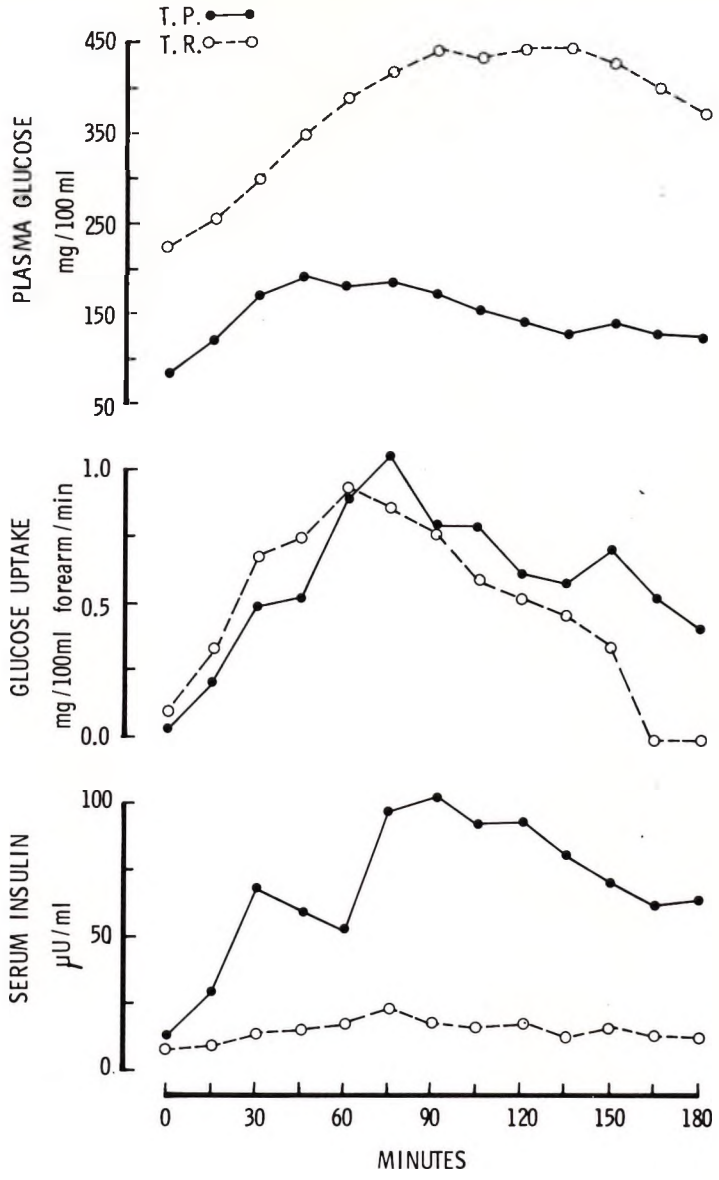

FIG. 31 Comparison of the response following 100 g oral glucose load in diabetic T.R. (Table 31) and T.P. - a 47 year old normal male

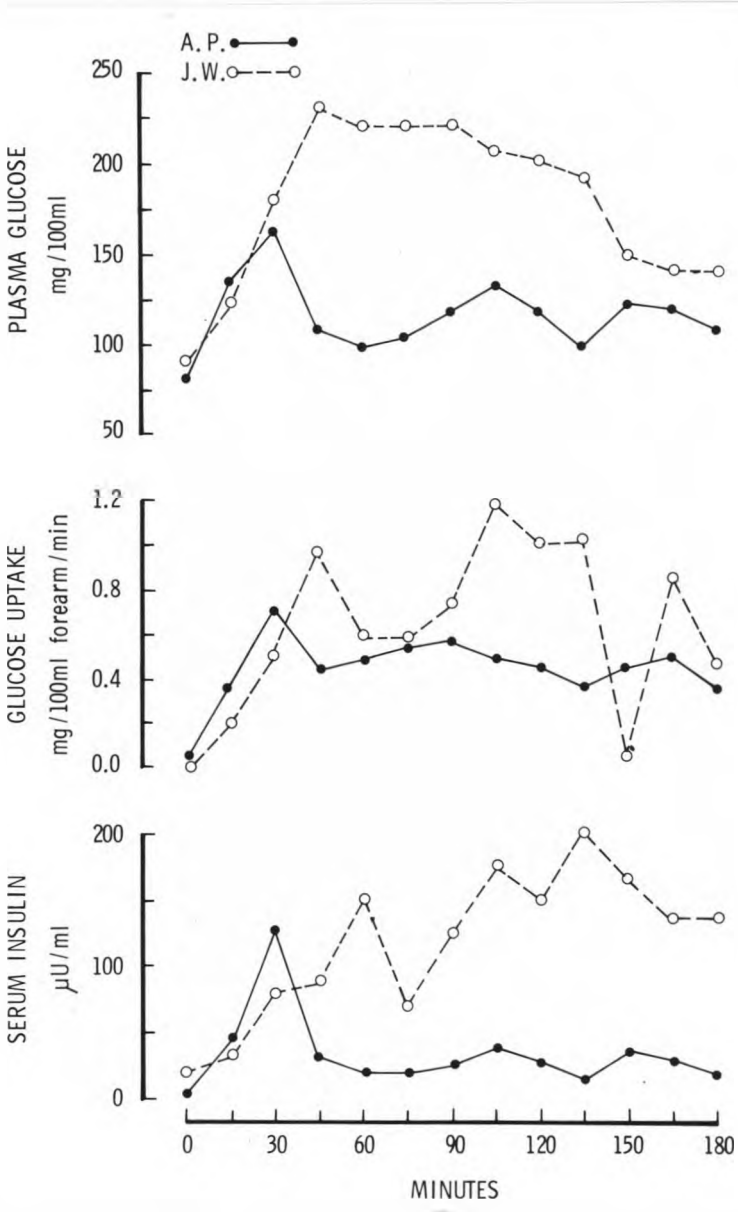

FIG. 32 Comparison of the response following 100 g oral glucose load in diabetic J.W. (Table 31) and A.P. - a 41 year old normal male

were 218 mg/100 ml in T.R. but only 56 mg/100 ml in T.P. In J.W. (Fig. 32) the increment in FGU (0-180 min) was 118 mg/100 ml forearm compared with 74 mg/100 ml forearm in A.P., yet in J.W. the incremental area under the glucose curve was 1.6 fold greater than that in the normal subject.

DISCUSSION

The present findings (Figs. 26 and 27) show that in the diabetics carbohydrate restriction reduces basal glucose concentrations and lowers the glucose tolerance curve but does not influence overall glucose tolerance (incremental area 0-180 min). When the responses are analysed hour by hour, however, it emerges that the elevation in glucose concentrations in the third hour of the GTT was significantly increased by carbohydrate restriction (Fig. 27); on this basis glucose tolerance is not improved by the dietary change but, on the contrary, is reduced even further. Since the lowering of the glucose tolerance curve was not associated with improved glucose tolerance, it must be attributed entirely to the fall in the basal glucose concentration - a response which recent studies (Cahill et al., 1966; Owen et al., 1969; Paul and Bortz, 1969; Kreisberg et al., 1970) indicate to be essentially a reflection of an adaptive decrease in gluconeogenesis.

As such, these findings confirm previous results (Jackson et al., 1971) suggesting that in diabetics and non-diabetics, the effects of carbohydrate restriction on glucose tolerance differ not in nature but only in degree. Thus in each group there is both a fall in the basal glucose concentration and an impairment in the capacity for glucose disposal (Jackson et al., 1971; See Chapter 6, Fig. 15); however, since in diabetics, in contrast to non-diabetics,

the fall in basal glucose levels is the relatively greater change, an overall lowering of the glucose tolerance curve is the outcome.

Fig. 27 shows that the incremental glucose area 120-180 min was increased despite a significant rise in concomitant peripheral glucose removal. Thus in the light of the generally accepted view that ingested glucose is taken up in either liver or peripheral tissues(Cahill et al., 1959) the observations suggest that the further impairment of glucose tolerance following carbohydrate restriction is the result not of decreased peripheral glucose uptake, but of diminished hepatic glucose conservation (Madison, 1969).

From the current data the approximate disposition of the 100 g load in the body as a whole may be calculated [see Chapter 5, Results section (b)]. On a normal diet some 29.8 g and 26.1 g are accounted for by increased peripheral glucose uptake and hepatic glucose conservation respectively (Table 37) while after a low carbohydrate diet it is possible to account for the disposition of the entire glucose load without invoking any participation by the liver in glucose conservation. Thus while peripheral glucose uptake is quantitatively preserved and if anything enhanced by carbohydrate restriction (presumably on a basis similar to that postulated for carbohydrate-deprived non-diabetics as described in Chapter 6, see Discussion), the hepatic response to glucose ingestion is totally ablated.

Fig. 28 shows that blood lactate concentrations were increased by glucose ingestion before but not after carbohydrate restriction. Since the rise in lactate concentrations after glucose loading appears to reflect increased hepatic glucose uptake (see Chapter 5, Discussion), the present responses support the conclusion that hepatic glucose uptake during the GTTs was markedly impaired following the low carbohydrate diet. It is clear, furthermore, that qualitatively

TABLE 37

Suggested "balance sheet" for the disposition of 100 g glucose following oral administration before and after carbohydrate restriction in diabetics

	Before	After
	g	g
Total peripheral glucose uptake	29.8	42.3
Total hepatic glucose conservation	26.1	0.0
Remaining in glucose space after 180 min	30.4	51.2
Loss in urine	13.7	6.5
	100.0	100.0

the influence of carbohydrate restriction on the lactate and ketone body responses to glucose loading (Figs. 28 and 29) is similar in diabetics and non-diabetics (see Chapter 6, Figs. 19 and 20) and the implications of these findings are discussed in Chapter 6. Since lactate uptake in muscle is determined mainly by the arterial lactate concentration (Jorfeldt, 1970), the failure to find significant AV-MV lactate differences across the forearm during the GTT before carbonydrate restriction is probably due to the fact that, as a result of the block to hepatic glucose uptake, AV lactate levels rose only insubstantially after glucose loading.

Although sufficient results are not yet available which allow

a meaningful comparison of GTT data between diabetics and non-diabetics on a normal diet, certain tentative conclusions may be drawn. Fig. 30 contrasts the response of K.P. (Table 31) with an age-matched normal male subject (J.B.) and shows that despite greater elevations in peripheral glucose removal in K.P. at both 90 and 180 min, glucose concentrations in the diabetic rose by increments 5-7 fold greater than those in the non-diabetic. Similar remarks apply to the results shown in Figs. 31 and 32. Thus although for any given plasma glucose concentration less glucose is utilised by peripheral tissues in the diabetics, the absolute amounts taken up within the context of the GTT were not reduced in the diabetics. The results suggest, therefore, that in the former decreased glucose conservation by non-peripheral tissues, i.e. the liver, constitutes the major underlying disturbance. More specifically it may be concluded that since in non-ketotic diabetics the ready suppressibility of hepatic glucose output by glucose and insulin administration appears to be retained (Bondy et al., 1949b; Forbath and Hetenyi, 1966), it is the failure to augment hepatic glucose utilisation after glucose loading which is the major cause of the reduction in hepatic glucose conservation and hence, in glucose tolerance.

The foregoing is consistent with several previous reports. Thus, the central role of the liver as a major determinant of glucose tolerance was emphasised over 30 years ago by Soskin et al. (1934) who found in dogs that the production of a normal glucose tolerance curve, while not dependent on an intact pancreas, was dependent on the presence of a normal liver. Ashmore et al. (1957) showed in vitro that both glucose phosphorylation and the rate of glucose degradation along glycolytic pathways was strikingly reduced in the diabetic liver. Madison et al. (1960, 1962) demonstrated in vivo that the response of the liver to a glucose load is impaired in diabetic

animals; thus, although during glucose infusions hepatic glucose storage began at arterial glucose concentrations of 116 mg/100 ml in normal dogs, this occurred in diabetic animals only when concentrations reached 200 mg/100 ml and in the more severely diabetic dogs, not at all, despite the elevation of arterial glucose concentrations to levels approaching 500 mg/100 ml. These observations are consistent with more recent studies by Perley and Kipnis (1967) which show that whereas in normal subjects a glucose infusion of only 31 g is sufficient to simulate the 100 g oral glucose tolerance curve, some 50 g is required in non-obese diabetics. These latter workers concluded that in diabetics a greater than normal proportion of ingested glucose reaches the peripheral circulation and that this is the consequence of a reduction in hepatic glucose extraction. Finally, the current proposals are supported by the numerous studies of enzyme activity which indicate that in diabetic liver glucose utilisation is markedly reduced (Cahill et al., 1959; Ferris, 1964; Steiner, 1966; Weber et al., 1966; Gold, 1970).

In contrast to the present findings (Figs. 30-32), reduced peripheral glucose uptake during GTTs has been reported in diabetics after a normal diet by Butterfield and Whichelow (1965, 1971). These observations (Butterfield and Whichelow, 1965, 1971) are difficult to interpret, however, since the authors did not measure insulin response nor did they distinguish between insulin-requiring and non-insulin requiring diabetics in the treatment of the data. It is conceivable, therefore, that while in some patients peripheral glucose uptake was reduced, in others it was equal to or greater than normal. Indeed, previous studies imply that this is the case. Thus while after glucose challenge A-V glucose differences across the forearm were greatly diminished or absent in some diabetics (Holst, 1922; Lawrence, 1924; Lundsgaard and Holbøll, 1925; Rabinowitch, 1927; Glassberg, 1930), Somogyi (1948) concluded that most patients exhibited

A-V differences as great as and often greater than those in normal subjects. Indeed the largest A-V difference reported by Mosenthal and Barry (1946) was 102 mg/100 ml in a diabetic 30 min after glucose ingestion. Cavett and Seljeskog (1933) on the other hand found that A-V differences were similar in diabetics and non-diabetics.

Soskin and Levine (1952) have emphasised that although peripheral glucose assimilation in the absence of insulin is markedly decreased, this defect may be largely overcome by increasing blood glucose concentrations. Indeed, in all but the most severe states of insulin deficiency a new equilibrium is reached with essentially normal rates of peripheral glucose assimilation - albeit obtained at much higher blood glucose concentrations (Soskin and Levine, 1937; Soskin and Levine, 1952; Issekutz, 1958). These increased levels of blood glucose are provided at the expense of increased hepatic glucose production. Although, as acknowledged above, the cellular uptake of glucose is impaired in diabetics, the conclusion of Soskin and Levine (1937, 1952)

> "When, however, one compares the rate of utilisation of the normal animal at its usual normal blood sugar levels with the rate of utilization of the diabetic animal at the hyperglycaemic levels which it ordinarily maintains, it is apparent that the diabetic animal habitually uses as much or more sugar than the normal animal",

is still valid not only in eviscerated normal and depancreatised dogs but in normal and diabetic humans as well. Thus, a homeostatic control mechanism is operating in a sense

> "When the ability of the tissues to use carbohydrate is impaired as by deficiency of insulin, impairment of insulin action... the head of pressure of blood sugar is raised, thereby facilitating the ability of the tissues to use sugar",

as suggested by Himsworth (1939) in his Goulstonian lectures and demonstrated by Wrenshall et al. (1964).

It has been demonstrated that glucose absorption by the small

intestine is enhanced in human diabetics (Vinnik et al., 1965) and this finding is supported by several studies in diabetic rats (Crane, 1961; Aulsebrook, 1965; Crane, 1968; Flores and Schedl, 1968; Olsen and Rosenbert, 1970). Others (Levinson and Englert, 1970), however, have failed to find any consistent increase in sugar absorption in diabetic over normal rats. Nevertheless the question arises as to whether an increased rate of absorption could play any role in accentuating the impairment of glucose tolerance which characterises diabetes. While this question cannot be answered with certainty until further information becomes available, it seems unlikely at this point that an increase in absorption of the order reported (Vinnik et al., 1965) would have anything more than a marginal influence on the glucose tolerance curve (Holdsworth, 1969).

SUMMARY

The relationship between the basal glucose concentration, glucose tolerance and peripheral glucose uptake has been studied in non-obese, non-insulin requiring diabetic males by determining FGU during 100 g GTTs before and after carbohydrate restriction.

On a normal diet basal glucose concentrations were elevated and glucose tolerance was grossly impaired; the increment in FGU during the GTT (0-180 min) amounted to 52.8 mg/100 ml forearm. After carbohydrate restriction basal glucose concentrations were reduced, the glucose tolerance curve was lowered and the increment in FGU rose to 76.5 mg/100 ml forearm; glucose tolerance, however, expressed as the incremental area under the glucose tolerance curve (0-180 min), remained unchanged.

Serum insulin responses during the GTTs were low and uninfluenced by carbohydrate restriction. Blood lactate concentrations were

increased by glucose loading before but not after carbohydrate restriction. FFA and β-hydroxybutyrate concentrations fell progressively after glucose loading while conversely, acetoacetate concentrations were initially unchanged. During the GTT acetoacetate was taken up by the forearm while lactate and β-hydroxybutyrate were released.

In addition, the response of three diabetics on a normal diet were compared with those of age-matched normal men; in each diabetic FGU was equal to or greater than that in the normal subjects, but despite this the increment in glucose concentrations was several fold greater.

The results suggest that in these diabetics (a) the lowering of the glucose tolerance curve by carbohydrate restriction is not synonomous with an overall improvement in tissue glucose disposal but is due primarily to a fall in the basal glucose concentration and (b) the impairment of glucose tolerance both before and after carbohydrate restriction is predominantly the result of a reduction in hepatic rather than peripheral glucose uptake.

CHAPTER 8

THE RESPONSE TO GLUCOSE LOADING IN YOUNG MEN WITH MYOCARDIAL INFARCTION: PRELIMINARY RESULTS AND TENTATIVE CONCLUSIONS

INTRODUCTION

Although the metabolic events resulting in atherosclerosis remain obscure, an association between ischaemic heart disease and disturbances in carbohydrate metabolism is widely recognised. It is known that diabetes is more prevalent in patients with myocardial infarction (M.I.) than in normal subjects while M.I. occurs more frequently in diabetics than in nondiabetics (Clawson and Bell, 1949; Bell, 1952; Sievers et al., 1961; Keen et al., 1965; Ostrander et al., 1965; Wahlberg and Thomasson, 1968). Of particular interest, however, is the finding of an increased insulin response to glucose loading reported in a proportion of nondiabetics with myocardial infarction and peripheral vascular disease (Peters and Hales, 1965; Nikkila et al., 1965; Welborn et al., 1966; Tzagournis et al., 1967; Christiansen et al., 1968; Sloan et al., 1970; Malherbe et al., 1971), since this raises the possibility that by promoting lipid accumulation in arterial intima, hyperinsulinism may play a cardinal role in the pathogenesis of atherosclerosis (Mahler, 1965; Stout, 1968, 1969; Kuo, 1969).

The aim of the present study was to determine whether hyperinsulinism was indeed a feature after oral glucose loading in young men with M.I. and, if so, whether this response could be attributed to an increase in peripheral insulin resistance.

SUBJECTS AND METHODS

100 g oral GTT's were carried out on 22 nonobese, nondiabetic men who had suffered a M.I. at or before the age of 41 years (Table 38). All subjects gave their informed consent for the investigation. Young men were specifically selected for study since it was reasoned that if disturbed glucose homeostasis is indeed important in

TABLE 38

Clinical data

Subject	Age	Height	Weight	Family history of myocardial infarction (M.I.) or diabetes (D)	Age of first attack
	yr	cm	kg		yr
W.G.	41	183	79.3	-	40
A.P.	44	170	73.5	-	40
L.E.	41	175	76.2	-	39
G.M.	33	173	79.9	-	32
R.A.	32	175	84.0	M.I.	28
R.S.	40	173	78.0	-	38
F.N.	44	174	84.6	M.I.	39
A.C.	40	186	85.6	M.I.	40
F.A.	36	169	65.2	-	35
E.L.	40	179	66.8	-	40
S.B.	43	173	69.3	-	40
S.D.	42	168	65.7	-	40
R.L.	40	178	76.0	-	37
H.S.	43	164	70.5	-	30
B.M.	42	172	62.6	-	41
W.T.	39	186	87.5	-	37
I.R.	36	183	87.1	-	33
G.N.	42	180	72.8	-	40
K.B.	38	175	83.1	D	37
G.B.	40	173	85.0	-	39
D.B.	31	183	84.0	-	28
D.T.	37	183	81.6	D	33
Mean	39.0 ± 0.8*	17o ± 1	77.2 ± 1.7		36.6 ± 0.9

* Standard error of mean

promoting early coronary atherosclerosis, the abnormalities would be most marked and thus most readily detectable in those with premature disease. Patients currently taking either clofibrate or propranolol were excluded from the study (Kotler et al., 1966); application of these criteria however resulted in the rejection of only six patients. Only those subjects with indisputable electrocardiographic evidence of M.I. were accepted for study; the changes in serum enzymes, when available, were always consistent with the diagnosis. No subject had any abnormality in hepatic, cardiac or renal function and did not suffer from any systemic or endocrine disorder. All subjects consumed a minimum of 220 g carbohydrate daily for at least seven days prior to the performance of a GTT, the latter being carried out at an interval not less than three months following the most recent M.I.

RESULTS

The results to be described represent a comparison of the responses in the M.I. group (Table 38) and the normal subjects described in Chapter 5. Because these two groups are not accurately matched for age and weight (Table 38) and because work is still in progress aimed at collecting data on suitably matched normal subjects, the present results must be regarded as preliminary and the conclusions tentative. The indications are, however, that the current conclusions will not require modification even when the necessary results have been collected. For this reason and because at the time of writing, direct studies of peripheral glucose uptake have not been reported in patients with M.I., the available data are reported as preliminary results; furthermore the age differences between the M.I. and control groups are small, and, indeed, insufficient to account for the

differences in response outlined below (see DISCUSSION).

Figs. 33 and 34 (Table 39) summarise the responses of the normal and M.I. groups during the GTTs. In the M.I. group the rise in AV glucose concentrations during the first half hour after glucose loading was similar to that in normal subjects but whereas in the latter the 30 min value represented the peak response, levels in the M.I. group continued to rise steeply for a further 15 min and by 60 min had fallen only marginally from peak concentrations. AV glucose concentrations were significantly higher in the M.I. group during the periods 45-75 and 120-165 min. Serum insulin responses were similar in the two groups and although in the M.I. patients mean concentrations were higher from 45-75 min, these differences did not achieve statistical significance. FGU in the M.I. group was significantly greater than that in normal subjects between 45-75 min, at 120 min and at 135 min. Insulin removal by the forearm tissues was demonstrable during the GTTs in the M.I. patients (Fig. 35; Table 40) as in normal subjects (see Chapter 5, Fig. 11). Insulin half-life was not significantly different in the normal and M.I. groups (Table 41). The increment in FGU from 0-180 min (calculated as the incremental area under the curve) was greater in the M.I. group than in normal subjects ($p < .02$), being 98.9 and 74.3 mg/100 ml forearm respectively; both peripheral insulin responsiveness ($\Delta FGU/\Delta insulin$) and β-cell sensitivity to insulin ($\Delta insulin/\Delta glucose$) were normal in the M.I. group (Table 42). Mean ($\pm$ SEM) forearm blood flow during the GTTs was not significantly different in the M.I. and normal subjects, being 2.8 $\pm$ 0.2 and 3.4 $\pm$ 0.2 ml/100 ml forearm/min. respectively. FFA and growth hormone responses were similar in the two groups. Circulating lipid levels were higher in the M.I. group (Table 43), but among the individual patients fasting trigyceride concentrations were not correlated with either the total insulin or

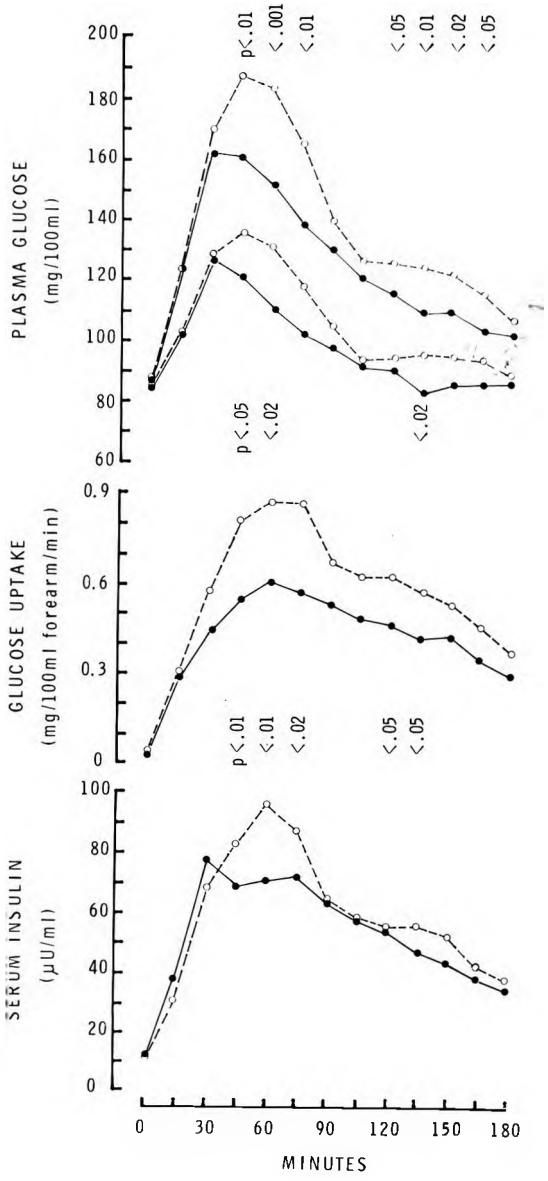

FIG. 33 Comparison of mean response following 100 g oral glucose load in 25 normal subjects (●—●) and 22 men with myocardial infarction (o--o). With respect to glucose concentrations - the upper glucose curves denote levels in arterialised venous blood, the lower curves levels in mixed venous blood; the upper row of p values refer to differences in arterialised venous levels, the lower row to differences in mixed venous levels

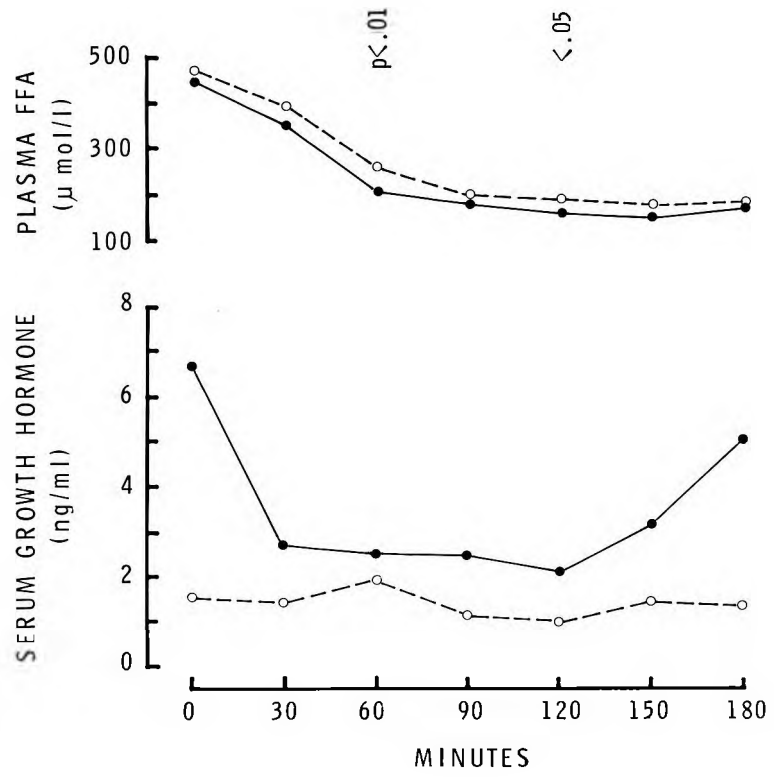

FIG. 34 Comparison of mean response following 100 g oral glucose load in 25 normal subjects (●—●) and 22 men with myocardial infarction (o--o)

glucose responses during the GTTs (expressed as incremental areas 0-180 min). The disposition of the ingested glucose load has been calculated (Table 44) as described in Chapter 5.

DISCUSSION

The present findings show that the rise in plasma glucose concentrations after oral glucose loading is both significantly greater and more prolonged in men who have suffered a M.I. than in normal subjects (Fig. 33) and as such are consistent with several previous reports (Cohen and Shafrir, 1965; Nikkila et al., 1967;

TABLE 39

Mean response following 100 g oral glucose load in 22 men with myocardial infarction

		Minutes						
		0	15	30	45	60	75	90
Glucose mg/100 ml	AV*	88 ± 1**	124 ± 4	170 ± 4	187 ± 5	183 ± 6	165 ± 7	140 ± 6
	p §	N.S.	N.S.	N.S.	<.01	<.001	<.01	N.S.
	MV ‡	86 ± 1	103 ± 2	129 ± 3	136 ± 5	131 ± 5	118 ± 6	105 ± 6
	p	N.S.	N.S.	N.S.	<.05	<.02	N.S.	N.S.
Glucose uptake mg/100 ml forearm/min		.045 ± .007	.300 ± .032	.579 ± .053	.810 ± .075	.871 ± .084	.866 ± .102	.676 ± .095
	p	N.S.	N.S.	N.S.	<.01	<.01	<.02	N.S.
Insulin µU/ml		12 ± 1	32 ± 3	69 ± 6	83 ± 8	95 ± 10	88 ± 8	65 ± 6
	p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
FFA µmol/l		464 ± 22	–	390 ± 20	–	254 ± 16	–	197 ± 5
	p	N.S.		N.S.		<.01		N.S.
Growth hormone ‡ ng/ml		1.47 ± 0.4	–	1.4 ± 0.3	–	1.9 ± 0.9	–	1.1 ± 0.1
	p	N.S.		N.S.		N.S.		N.S.
Blood flow ml/100 ml forearm/min		3.3 ± 0.3	2.5 ± 0.2	2.6 ± 0.2	2.9 ± 0.3	3.1 ± 0.2	3.4 ± 0.3	3.5 ± 0.3
	p	<.02	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Arterialised venous
** Standard error of mean
§ Significance of difference from mean normal response (Table 16, Chapter 5)
‡ Mixed venous
‡ n = 10

TABLE 39 Cont.

Mean response following 100 g oral glucose load in 22 men with myocardial infarction

		Minutes					
		105	120	135	150	165	180
Glucose mg/100 ml	AV*	127 ± 5**	126 ± 3	125 ± 3	122 ± 3	116 ± 3	107 ± 4
	p §	N.S.	< .05	< .01	< .02	< .05	N.S.
	MV ‡	94 ± 5	95 ± 4	96 ± 4	95 ± 3	94 ± 3	89 ± 3
	p	N.S.	N.S.	< .02	N.S.	N.S.	N.S.
Glucose uptake mg/100 ml forearm/min		.631 ± .066	.630 ± .070	.579 ± .056	.532 ± .050	.454 ± .052	.374 ± .051
	p	N.S.	< .05	< .05	N.S.	N.S.	N.S.
Insulin μU/ml		58 ± 7	56 ± 5	56 ± 5	53 ± 4	43 ± 4	38 ± 3
	p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
FFA μmol/l		–	190 ± 2	–	179 ± 4	–	183 ± 3
	p		< .05		N.S.		N.S.
Growth hormone ‡ ng/ml		–	1.0 ± 0.0	–	1.5 ± 0.4	–	1.4 ± 0.4
	p		N.S.		N.S.		N.S.
Blood flow ml/100 ml forearm/min		3.7 ± 0.4	3.7 ± 0.3	3.9 ± 0.4	3.7 ± 0.3	3.8 ± 0.3	3.9 ± 0.3
	p	N.S.	N.S.	< .02	N.S.	N.S.	N.S.

* Arterialised venous
** Standard error of mean
§ Significance of difference from mean normal response (Table 16, Chapter 5)
‡ Mixed venous
= n = 10

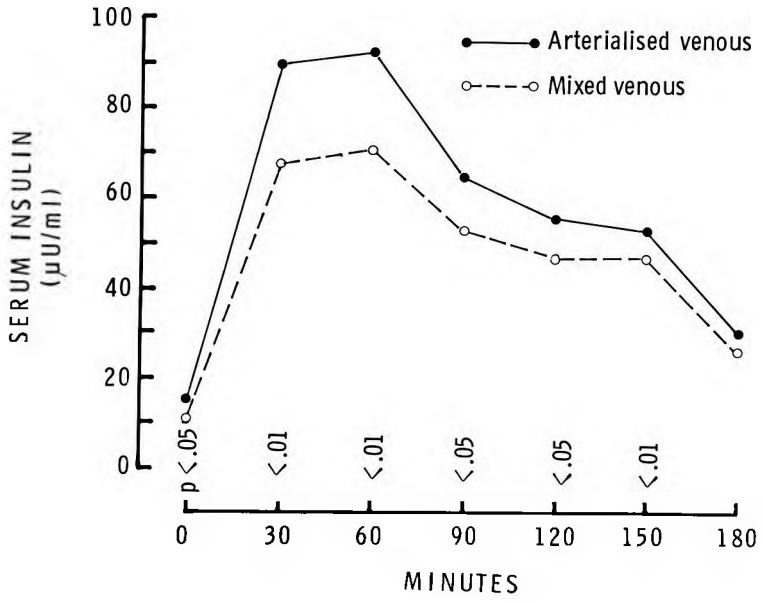

FIG. 35 Mean changes in insulin concentrations following 100 g oral glucose load in five men with myocardial infarction

Tzagournis et al., 1968; Sloan et al., 1970; Matherbe et al., 1971). More importantly, however, the concomitant elevation in EGU in the M.I. group is also significantly increased - an increase which, since corresponding insulin levels were not elevated, must be attributed to the higher circulating glucose concentrations (Lundsgaard et al., 1939; Park et al., 1959; Randle, 1963). These results suggest that the greater rise in glucose concentrations in the M.I. group cannot be the consequence of decreased peripheral glucose utilisation. Thus, since the disposition of ingested glucose is shared predominantly between peripheral tissues on the one hand and the liver on the other (Cahill et al., 1959; Perley and Kipnis, 1967), it may be concluded that in the M.I. group reduced hepatic glucose uptake is the major metabolic abnormality (Table 44) and that if, indeed, insulin resistance is increased in this group, it would seem to be at

TABLE 40

Mean serum insulin concentrations (μU/ml) in arterialised venous (AV) and mixed venous (MV) blood following 100 g oral glucose load in five men with myocardial infarction

	Minutes						
	0	30	60	90	120	150	180
AV	15 ± 1*	90 ± 10	93 ± 4	65 ± 13	56 ± 8	53 ± 8	30 ± 5
MV	11 ± 1	68 ± 12	71 ± 7	53 ± 11	47 ± 7	47 ± 7	26 ± 3
p**	<.05	<.01	<.01	<.05	<.05	<.01	N.S.

* Standard error of mean

** Significance of difference between means

TABLE 41

Disappearance of exogenous insulin from serum

	Basal insulin	$T_{\frac{1}{2}}$	$T_{\frac{1}{2}}$ minus basal insulin concentration	Time before linearity
	μU/ml	min	min	min
Normal subjects*	10 ± 2**	12.2 ± 0.8	9.6 ± 0.5	15.7 ± 1.6
Myocardial infarction group*	9 ± 1	11.5 ± 0.6	9.6 ± 0.5	15.0 ± 1.8
p	N.S.	N.S.	N.S.	N.S.

* n = 7 for each group

** Standard error of mean

TABLE 42

Comparison of responses* following 100 g oral glucose load

in 25 normal subjects and 22 men with myocardial infarction

	Normal subjects	Myocardial infarction group	p
Glucose** units	125 ± 6§	157 ± 8	<.01
FGU mg/100 ml forearm	74.3 ± 4.7	98.9 ± 9.0	<.02
Insulin units	132 ± 14	146 ± 12	N.S.
△FGU/△Insulin	.56 ± .05	.68 ± .09	N.S.
△Insulin/△Glucose	1.06 ± 0.1	0.93 ± .09	N.S.

* Expressed as △ i.e. incremental area under response curve 0-180 min

** Refers to concentrations in arterialised venous blood

§ Standard error of mean

TABLE 43

Comparison of fasting lipid concentrations (mg/100 ml) in 25 normal subjects and 22 men with myocardial infarction

	Normal subjects	Myocardial infarction group	p
Plasma triglyceride	84 ± 42*	125 ± 65	$<.02$
Serum cholesterol	194 ± 32	257 ± 54	$<.001$

* Standard error of mean

hepatic rather than peripheral level.

Several authors (United States Department of Health, Education and Welfare, 1963, 1964; Butterfield, 1964; West et al., 1964; Hayner et al., 1965; Chlouverakis et al., 1967; Boyns et al., 1969; Lauvaux and Staquet, 1970) have demonstrated an increase with age in the glucose concentrations observed after glucose loading but that, despite this, the timing of the peak glucose level does not change (Butterfield, 1964). In view of the latter and because the age discrepancy between the control and M.I. groups was too small to account for the magnitude of the differences in glucose levels observed, the present data would appear to represent real metabolic differences between the groups.

Previous workers (Tzagournis et al., 1967; Malherbe et al., 1971) have found that patients with ischaemic heart disease achieve not only higher glucose concentrations but higher insulin levels as well,

TABLE 44

Comparison of disposition of 100 g glucose following oral administration in normal subjects and men with myocardial infarction

	Normal subjects	Myocardial infarction group
	g	g
Total peripheral glucose uptake	43.2	61.0
Hepatic glucose conservation	53.4	34.6
Loss in urine	0.0	0.0
Remaining in glucose space after 180 min	3.4	4.4
	100.0	100.0

The latter could not be confirmed statistically in the current study and there is at present no adequate explanation for this discrepancy between the current findings and those of the previous workers noted above. It is conceivable, however, that the fundamental metabolic disturbances in our patients and those studied by Tzagournis et al. (1967), Malherbe et al. (1971) and Sloan et al. (1970) differ not in nature but only in degree. Thus the gradual development of a failure in hepatic insulin responsiveness would, by progressively reducing hepatic glucose extraction, allow increasingly greater than normal proportions of ingested glucose to reach the peripheral circulation. By virtue of the occurrence of "inappropriately" high glucose concentrations in the general

circulation the β-cell "glucostat" would become reset at a higher level so that a standard glucose challenge would habitually call forth an enhanced insulin response. Possibly then, this patient group may represent a phase in the evolution of the disease process earlier than that prevailing in other patient groups (Tzagournis et al., 1967; Malherbe et al., 1971), in whom the higher insulin levels reflect islets already fully adapted to a greater secretory response.

In any event it is clear that in the present M.I. group marked abnormalities in the regulation of carbohydrate metabolism were not a feature at the time of the first attack and accordingly a major role for disturbed glucose homeostasis in the etiology of coronary atherosclerosis must be seriously questioned. This derives particular emphasis from the fact that the patients were men with premature disease in whom unequivocal abnormalities in insulin concentrations would have been anticipated had their predisposition to M.I. been dependent to any significant degree on hyperinsulinism. It must be borne in mind that atherosclerosis is probably the end result of multiple and long-continued metabolic events. The present comments, therefore, can be applied only to the particular patient group reported here. Further studies in other patient groups are required before the current confusion surrounding the subject of atherogenesis can be significantly lessened.

SUMMARY

FGU was studied during 100 g GTTs in nonobese, nondiabetic men who had suffered M.I. at or before the age of 41, and the results compared with the response in normal subjects.

In the M.I. group the rise in plasma glucose concentrations was greater in magnitude and longer in duration than that in normal subjects. Concomitant PGU was also significantly enhanced while the serum insulin responses did not differ from those of the control group. FFA and growth hormone responses were similar in normal subjects and patients with M.I.

The results show that the greater rise in glucose concentrations in the M.I. group is not the consequence of decreased peripheral glucose utilisation, and suggest that a reduction in hepatic glucose uptake constitutes the major metabolic abnormality. The data show, furthermore, that after glucose loading an increased insulin response was not a feature in the M.I. group and in these patients peripheral insulin resistance was not increased.

Further studies are required to elucidate the metabolic disturbances predisposing to the development of atherosclerosis.

CHAPTER 9

THE ORAL GLUCOSE TOLERANCE CURVE IN THE LIGHT OF THE PRESENT DATA: CONCLUDING COMMENTS, FURTHER OBSERVATIONS AND RECOMMENDATIONS

(a) THE GLUCOSE TOLERANCE CURVE AS A WHOLE

The results show that in normal subjects glucose uptake by peripheral tissues accounts for the disposition of only 43.2% of a 100 g oral glucose load (Chapter 5, Table 20). Since, apart from peripheral tissues, the liver is the only site of major glucose disposition during the GTT (Cahill et al., 1959), it may be concluded that hepatic glucose conservation accounts for the disposal of the major fraction (53.4%) of a 100 g glucose load. Accordingly it would be anticipated as, indeed, the results suggest (Chapters 6 and 7) that the impairment of glucose tolerance which is found in nondiabetics deprived of carbohydrate and in diabetics is primarily the consequence of a reduction and/or delay in hepatic glucose conservation rather than any change in peripheral glucose uptake. This is not to imply, however, that in these groups of subjects changes in glucose metabolism do not occur in peripheral tissue - they do, but the influence of such changes on the shape of the glucose tolerance curve is minor relative to the influence exerted by variations in hepatic glucose uptake.

With regard to the interpretation of the glucose tolerance curve as a whole several factors must be borne in mind. Firstly, it must be recognised that while the incremental area under the arterial glucose tolerance curve serves as an index of the combined contributions to glucose disposal of both liver and peripheral tissues it does not distinguish between them. Thus there are situations as shown in the diabetics (Chapter 7) where the overall incremental area (0-180 min) remained statistically unchanged following carbohydrate restriction but where the individual contributions of liver and peripheral tissues were considerably altered. Secondly, it must be recognised that while a lowering of the glucose tolerance curve may be conditioned by a real improvement in the capacity of tissues for

glucose disposal, it may be the result merely of a marked reduction in the basal glucose concentration irrespective of any change in overall glucose disposal. The lowering of the curve produced in the diabetics by carbohydrate restriction was specifically due to the fall in the basal glucose level and, being unassociated with increased tissue glucose uptake, cannot be regarded as evidence of "improved" glucose tolerance. The rational interpretation of the glucose tolerance curve, therefore, demands the differentiation not only of events in the periphery from those taking place in the liver, but also of changes in the basal concentrations from those which become apparent only after glucose loading.

(b) THE COMPONENTS OF THE GLUCOSE TOLERANCE CURVE

Further comments are now in order regarding the significance of various components of the glucose tolerance curve.

Jacobsen (1913) demonstrated in normal subjects an appreciable rise in blood sugar concentrations within five min of glucose ingestion, the maximum level being usually attained in 30 min and a return to pre-ingestion levels occurring within 105 min. Cori (1925, 1926a,b) and Cori and Cori (1926, 1927a,b,c) concluded that the initial rise of arterial and venous blood sugar levels after glucose ingestion is an expression of sugar absorption from the alimentary tract and that the subsequent reduction of blood sugar to normal levels is the result of two factors:- (1) the removal of sugar by the liver to form glycogen and (2) the removal of sugar by extrahepatic tissues, especially muscle, for the formation of tissue glucogen. Foster (1923b) had earlier agreed that glucose absorption was the obvious cause of the rise in blood sugar but commented "it is not so clear why the curve should again fall to normal as rapidly as it does at

a time when the rate of absorption from the gut can scarcely be diminished".

MacLean and de Wesselow (1921) presented a comparison of the blood sugar curves of a normal man and of a severe diabetic after ingesting 50 g glucose. The curves both rose sharply by nearly the same amount for the first half hour. At this point the normal curve broke and soon began to fall rapidly to the fasting level while the curve of the diabetic continued to rise for 120 min or more i.e. "presumably as long as aborption continued". These authors (MacLean and de Wesselow, 1921) concluded that apparently, in the normal, some mechanism which was dormant for the first half hour had been awakened and intervened to check the rising hyperglycaemia; they argued that the presence of an excess of sugar in the circulation stimulated the mechanism which deals with sugar and assumed it to be glycogen synthesis largely in the liver. This is wholly consistent with the findings during oral GTTs of (1) Bornstein and Holm (1922) that whereas the blood sugar began to rise immediately after glucose ingestion, the rise in respiratory quotient exhibited a definite latent period of 30-60 min and (2) Cherry and Crandall (1937) and Jeffcoate and Moody (1969) since the former (Cherry and Crandall, 1937) reported in dogs that hepatic glucose uptake was maximal only after 30 min while the latter (Jeffcoate and Moody, 1969) demonstrated in rats that maximal increments in liver glycogen levels occurred only after 20-60 min.

The present data support and throw further light on the concepts *that the ascending limb of the glucose tolerance curve is a reflection mainly of absorption and that a mechanism for glucose disposal exists which remains dormant for the first 30 min but which, once awakened, is responsible for the break in the curve* thereafter. *Table 45 shows that the rate at which plasma glucose concentrations*

TABLE 45

Increment in glucose concentrations (mg/100 ml) in arterialised venous plasma during 100 g oral glucose tolerance tests

	Dietary Carbohydrate	Minutes							
		0–15	15–30	30–45	45–60	60–75	75–90	90–105	105–120
Normal subjects*	Normal	38	38	-	-	-	-	-	-
Normal subjects**	Normal	41	42	-	-	-	-	-	-
	Low	42	50	36	3	-	-	-	-
Diabetics §	Normal	37	53	40	32	12	16	-	-
	Low	37	53	41	28	25	19	15	13

* Data derived from Table 16 (Chapter 5)

** Data derived from Tables 22 and 23 (Chapter 6)

§ Data derived from Tables 32 and 33 (Chapter 7)

rise within the first 15 min of glucose ingestion is remarkably constant irrespective of previous diet or diabetic status. This suggests that the increment in glucose levels during the initial 15 min is determined by a process uninfluenced to any major degree by insulin action i.e. intestinal absorption.

In the period 15-30 min differences between the groups become apparent, greater than normal increments in glucose levels being observed in carbohydrate-deprived nondiabetics and diabetics. Since the ascending limb of the arterial glucose curve reflects glucose entry into the general circulation, this suggests that while in the period 15-30 min absorption is still the major determinant of the curve increasing hepatic extraction is also a factor and that the latter is beginning to retard this rise significantly in normal subjects but not as effectively in carbohydrate-deprived nondiabetics or frank diabetics.

Evidence has been presented (Chapter 5) which strongly suggests that the failure of glucose levels to continue rising after 30 min in normal subjects (i.e. the break in the curve at 30 min) is due primarily to increasing hepatic glucose extraction rather than any change in peripheral glucose uptake. Indeed between 30-45 min glucose entry into the systemic vascular bed was 7.1 g less while peripheral glucose uptake was only 1.09 g more than in the preceding 15 min period (see Appendix, Table 5). The maintenance of the reduction in glucose entry into the general circulation after 30 min (Fig. 14) by a sustained high level of hepatic glucose extraction would appear to be, therefore, the principal factor responsible for the downward slope of the curve in the 30-45 min period and the continuing decline in glucose concentrations thereafter. The latter is consistent with the continuing rise in plasam lactate concentrations between 30-60 min which reflects enhanced hepatic glucose uptake, hepatic glycolysis and hepatic lactate release (See Chapters 5 and 6). It

would appear that in normal subjects the liver requires a period of "insulinisation" of 20-30 min before insulin action becomes fully manifest and that hepatic glucose uptake is in fact that mechanism referred to by MacLean and de Wesselow (1921) which remains dormant for the first 30 min and which is awakened only thereafter.

The rate of peripheral glucose uptake is fairly evenly distributed during the GTT in normal subjects being 13.0 g in the first hour, 17.5 g in the second hour and 12.7 g in the third hour (Chapter 5). Thus while increased peripheral glucose uptake clearly effects some reduction in glucose concentrations throughout the GTT and particularly after 30 min (Chapter 5, Table 16), it does not, unlike hepatic glucose uptake, significantly influence the shape of the glucose tolerance curve.

(c) THE INTERPRETATION OF THE GLUCOSE TOLERANCE CURVE

Five further points are worthy of note.

Firstly, it must be recognised that the response of the forearm tissues in terms of glucose uptake is influenced not only by the prevailing serum insulin concentration but also by circulating glucose levels. It follows then that estimates of FGU made during insulin infusion studies (during which regional concentrations of insulin, but not glucose, are increased) may not necessarily reflect the peripheral tissue response following glucose ingestion (when the tissues are exposed to elevated concentrations of both glucose and insulin). The contrasting patterns of FGU obtained in these two experimental situations after carbohydrate restriction bear this out (see Chapter 6, Figs. 15 and 18). Clearly, therefore, valid conclusions may be drawn only in the context of the techniques by which the results are obtained since the indiscriminate extrapolation of data from one

experimental situation to another which is not metabolically identical, serves merely to confuse and perpetuate error.

Secondly, it must be emphasised that the development of glycosuria during a GTT may occur in a normal subject and is not diagnostic of diabetes mellitus, even in the presence of a normal renal threshold for plasma glucose of 180 mg/100 ml (de Wardener, 1967; Robinson, 1967). The development of significant A-V differences across the forearm after glucose loading is the basis for this. Thus Fig. 36a (see Appendix, Table 16) shows in a normal subject that although AV glucose levels exceeded the renal threshold between 30-75 min, the MV glucose tolerance curve remained entirely within normal limits (Joplin and Wright, 1968; Reaven and Miller, 1968); a second GTT performed one week later (Fig. 36b; see Appendix, Table 16) provides further confirmation that this subject is indeed nondiabetic.

Thirdly, it must be borne in mind that a flat venous glucose tolerance curve is not necessarily indicative of glucose malabsorption (Holdsworth, 1969; Marks, 1969) and, indeed, may be found in entirely normal subjects in whom the forearm tissues are avidly extracting glucose from arterial blood (Fig. 37; see Appendix, Table 17). Thus, whereas in true malabsorption both the arterial and venous curves are flat, in normal subjects the arterial curve is never flat whatever the shape assumed by the venous curve. Furthermore, it is clear that by increasing the frequency of blood sampling from 30- to 15- min intervals, the appearance of flatness in the venous curve may be eliminated. Thus the sharp rise in venous glucose levels at 15 min (Fig. 37) is proof of rapid glucose absorption while the rather flat curve based only on half-hourly blood sampling is less convincing.

Fourthly, it is interesting to note the comments of Freinkel and Metzger (1969) regarding the persistence during a GTT of elevated

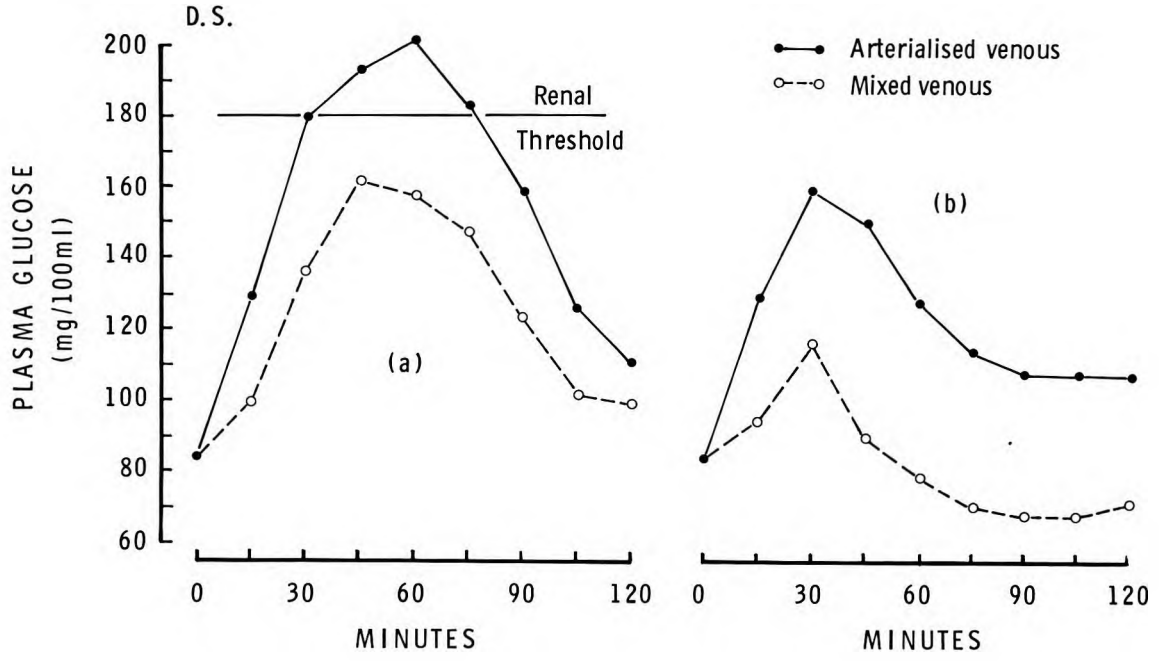

FIG. 36 Responses following 100 g oral glucose load observed one week apart in a normal subject (D.S.)

insulin concentrations at a time when venous glucose levels have returned to fasting values. They wrote "The significance of this phenomenon is unexplained". This phenomenon is readily explained, however, when it is appreciated that while venous glucose concentrations return to basal values after 120 min, arterial concentrations do not. Indeed, the arterial level remains elevated even at 180 min (see Chapter 5, Fig. 9) and it is the latter, not the venous level, which is the principle determinant of the prevailing insulin concentration.

Finally, it is evident that the return of MV glucose concentrations to basal levels is not necessarily an indication that disposal of the ingested glucose load has reached completion or that the metabolic response to the glucose load has ceased. Thus Fig. 9 (see Chapter 5) shows that although MV glucose levels had returned to baseline values by

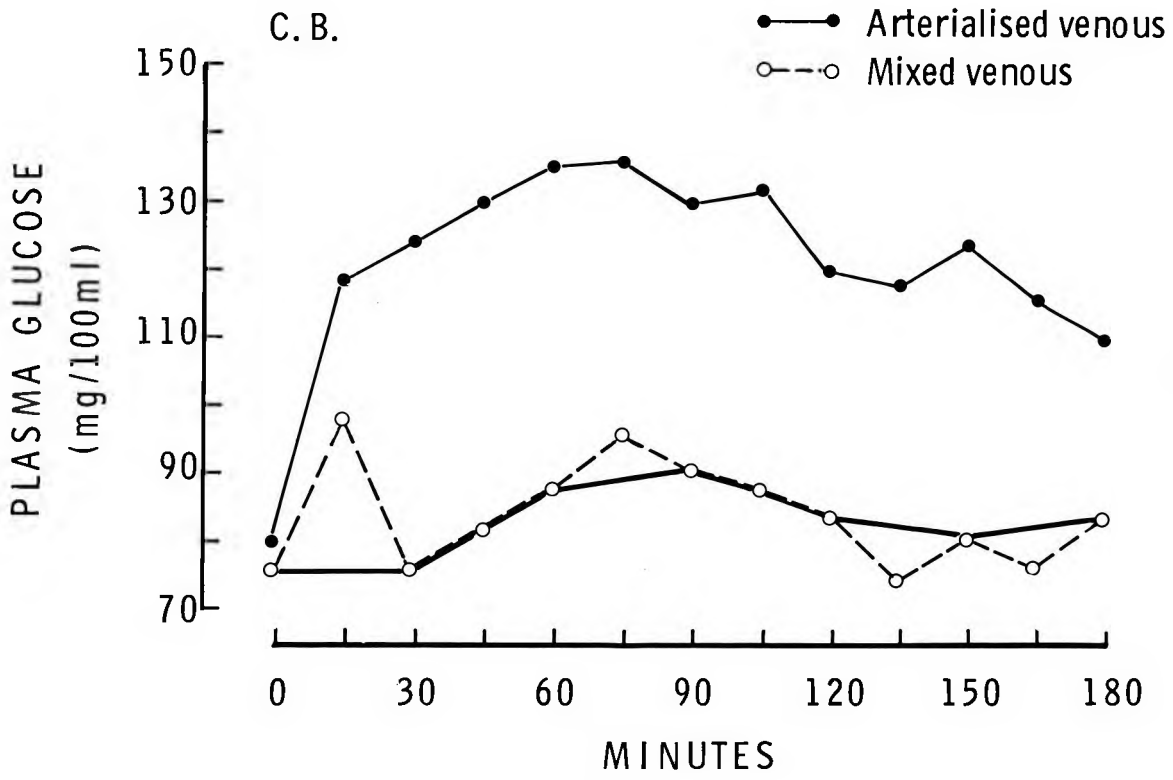

FIG. 37 Response following 100 g oral glucose load in a normal subject (C.B.) The solid line through the mixed venous curve illustrates the flat response obtained when concentrations are measured at half-hourly intervals only

135 min, AV glucose levels and serum insulin concentrations remained elevated even at 180 min and at this time FGU was still 10-fold that of pre-test levels.

(d) RECOMMENDATIONS REGARDING THE TECHNIQUE FOR PERFORMING THE ORAL GLUCOSE TOLERANCE TEST

It is of vital importance to recognise that inconsistencies and carelessness in technique may influence significantly the results obtained during GTTs - indeed, to the extent that a completely normal subject may

be misdiagnosed as prediabetic or mildly diabetic. Clearly, the psychological and medical implications of such an event for any one individual are such that errors of this nature must be avoided at all costs. The latter can be achieved, however, only by strict observance of certain requirements when carrying out the GTT.

The plasma glucose response to glucose ingestion may be varied artificially if, during the GTT, one and/or other of the following supervene:

(1) indiscriminate blood sampling from both arms or from different veins at the same or different levels of the same forearm

(2) sustained (i.e. for 30 min or longer) rises in forearm blood flow to levels 6.0 ml/100 ml forearm/min or more.

It has been emphasised by the present data (see Chapter 5, Table 16) and the findings of many workers (Somogyi, 1948; Mosenthal and Barry, 1950; Rabinowitz and Zierler, 1962a,b; Whichelow et al., 1967) how greatly the glucose concentrations of arterial, AV or superficial venous (SV) blood may exceed those in DV or antecubital MV blood after glucose loading. It is imperative, therefore, that consistency be maintained during a conventional GTT in sampling either antecubital MV blood which reflects predominantly regional muscle metabolism or blood from hand veins or superficial veins but not both. If a suitable antecubital mixed vein (e.g. vein B or vein C, Fig. 38) is chosen for sampling, then the curve obtained should be interpreted according to criteria for venous blood; if in contrast, blood is sampled from a superficial forearm vein (e.g. veins A, D or E, Fig. 38) or a vein on the back of the hand, the curve should be interpreted according to criteria for capillary blood. It is clear, furthermore, that if MV blood is chosen for sampling, proximal venous occlusion in order to aid sampling should be avoided; thus, by forcing blood from superficial to the deep venous bed, venous occlusion will artificially elevate the glucose concentrations in MV blood (Loughlin et al., 1943; Mottram and

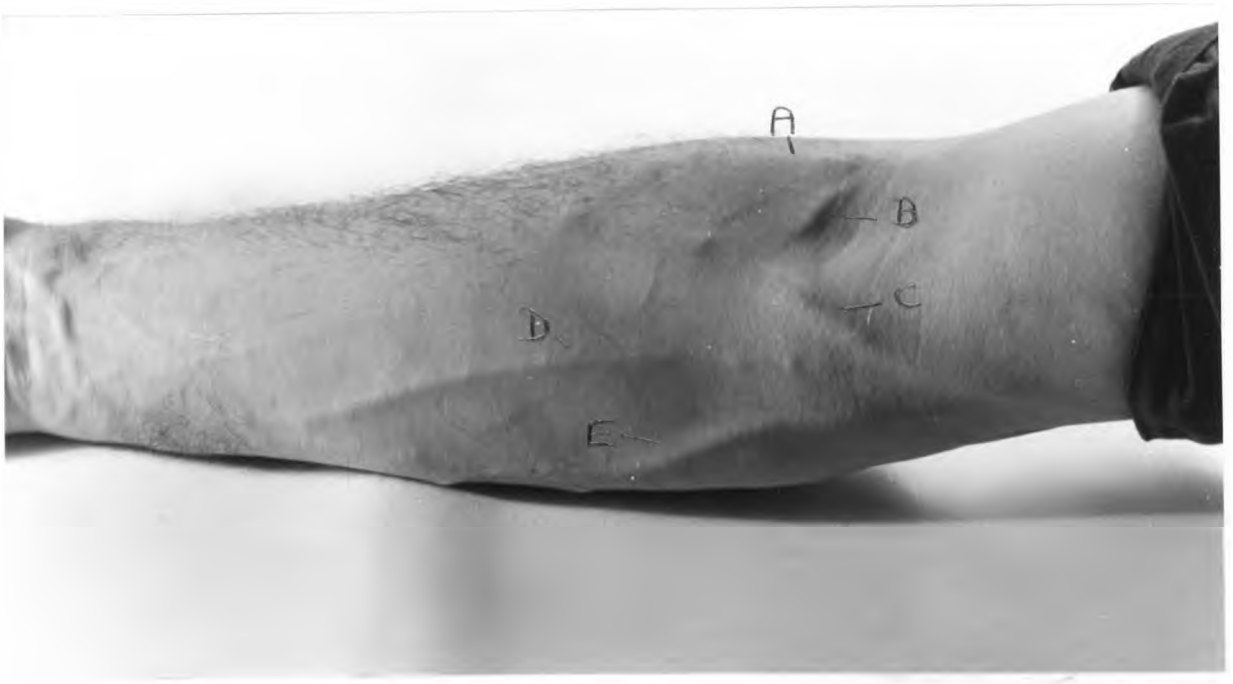

FIG. 38 Venous pattern in the right antecubital fossa of a normal subject

Butterfield, 1961).

Particularly when MV blood is chosen for sampling, situations associated with increased peripheral blood flow should be avoided since, for any given level of FGU, elevations in forearm blood flow will result in corresponding rises in the glucose concentrations of antecubital MV blood. The latter will then approximate arterial glucose levels more closely than they otherwise would, and the implications of this are mentioned above. The factors which may precipitate rises in forearm blood flow include the following: rises in the environmental temperature, changes in posture or exercise of the relevant forearm or body as a whole between the collection of blood samples, painful stimuli e.g. repeated venepuncture, nausea (associated with the ingestion of 100 g glucose), a full bladder (following the ingestion of 100 g glucose), and sudden noises e.g. ringing of the telephone, dropping of articles on the floor.

On the basis of the above considerations, it is recommended that

(1) the patient receives a detailed explanation of the purpose, significance and technique of the GTT well in advance of the actual performance of the test

(2) GTTs be performed in a quiet room, especially set aside for the purpose, in which the air temperature is maintained between 21-24°C

(3) the patient remains recumbent throughout the GTT

(4) an indwelling needle or catheter be employed rather than repeated venepuncture

(5) proximal venous occlusion be avoided during sampling

(6) rises in forearm blood flow be prevented by

(a) avoiding exercise of the forearm used for sampling prior to and during sampling particularly

(b) satisfying immediately any desire of the patient to micturate

(c) taking precautions in order to avoid the occurrence of sudden noise particularly during the 10 min immediately prior to sampling

(7) the GTT be repeated in the event of their being the slightest doubt regarding the acceptability of the conditions of the test.

Table 46 details the upper limits of the normal response during the 100 g oral GTT when all the above requirements are met.

TABLE 46

Upper limits (mean + 2 S.D.) of the normal response*

during 100 g oral glucose tolerance tests

	Minutes												
	0	15	30	45	60	75	90	105	120	135	150	165	180
Glucose AV mg/100 ml	98	158	204	215	213	197	174	151	146	140	148	140	136
Glucose MV mg/100 ml	94	124	161	171	167	151	136	128	125	112	114	120	116
Glucose uptake mg/100 ml forearm/min	.085	.707	.867	.907	1.027	1.084	.991	.874	.840	.798	.776	.641	.580
Insulin μU/ml	19	81	150	119	151	182	134	124	120	128	86	81	81

* Based on response in 25 normal subjects (see Chapter 15, Table 16)

APPENDIX

TABLE 1

Comparison of glucose concentrations (mg/100 ml) in arterial (A), arterialised venous (AV) and mixed venous (MV) plasma following 100 g oral glucose load

Time	J.M.			M.B.		
min	A	AV	MV	A	AV	MV
0	87	84	80	81	77	72
0	87	86	80	80	78	73
15	121	118	94	136	134	110
30	169	163	124	185	184	142
45	191	186	128	140	138	122
60	171	172	118	126	125	105
75	145	150	103	118	119	104
90	128	129	102	129	125	120
105	112	112	86	126	125	121
120	103	104	93	115	113	108
135	105	106	95	94	94	90
150	104	105	97	66	66	60
165	95	97	91	57	57	53
180	72	76	75	56	55	52

TABLE 2

Comparison of lactate concentrations (mmol/l) in arterial (A), arterialised venous (AV) and mixed venous (MV) plasma following 100 g oral glucose load

Time	T.P.		
min	A	AV	MV
0	0.73	0.76	0.73
30	0.84	0.81	0.72
60	0.99	1.03	0.84
90	0.87	0.90	0.92
120	0.78	0.80	0.76
150	0.60	0.62	0.62
180	0.58	0.58	0.63

TABLE 3

Incremental areas (Δ) under mean normal response curves for AV glucose (G), insulin (I) and forearm glucose uptake (U) following 100 g oral glucose load

Subject	ΔU	ΔI	ΔG	$\Delta U/\Delta I$	$\Delta I/\Delta G$
	units	units	units	units x 10^3	units x 10^2
M.R.	1.250	390	170	3.21	22.94
J.B.	0.624	100	125	6.24	8.00
C.B.	1.053	69	127	15.26	5.43
M.B.	1.257	70	146	17.96	4.79
P.L.	1.198	79	134	15.16	5.90
J.L.	1.625	175	122	9.29	14.34
G.M.	1.353	147	117	9.20	12.56
R.M.	0.553	123	111	4.50	11.08
D.S.	1.270	183	178	6.94	10.28
D.T.	1.713	62	113	27.63	5.49
A.Y.	1.357	236	117	5.75	20.17
C.K.	0.872	84	66	10.38	12.73
M.B.	2.006	144	114	13.93	12.63
D.M.	1.522	107	107	14.22	10.00
J.M.	1.928	114	150	16.91	7.60
C.H.	0.794	199	146	3.99	13.63
G.T.	1.298	95	151	13.66	6.29
B.S.	1.428	103	68	13.86	15.15
A.P.	1.211	94	121	12.88	7.77
J.S.	0.961	129	79	7.45	16.33
J.R.	1.503	94	151	15.99	6.23
R.H.	0.489	97	78	5.04	12.44
T.P.	1.629	171	197	9.53	8.68
C.D.	1.055	75	127	14.07	5.91
J.H.	1.021	160	122	6.38	13.11

TABLE 4

Raw data for correlation of arterialised venous insulin concentrations, arterialised venous-mixed venous insulin differences and forearm insulin uptake 30, 60 and 90 minutes following 100 g oral glucose load in 13 normal subjects

30 min			60 min			90 min		
AVI*	(AV-MV)I**	FIU§	AVI	(AV-MV)I	FIU	AVI	(AV-MV)I	FIU
92	20	27.4	120	36	30.6	150	50	55.5
43	3	5.0	60	14	18.3	50	12	19.1
40	15	17.7	57	12	19.3	56	13	20.9
48	13	14.2	72	15	20.1	77	15	24.0
280	50	138.5	56	0	0.0	61	19	31.4
115	15	32.6	150	27	34.0	174	36	37.8
72	20	29.4	78	13	26.7	60	0	0.0
160	76	82.9	180	70	85.5	110	35	42.8
100	26	29.6	105	17	26.5	66	8	13.4
130	38	124.0	85	0	0.0	83	19	47.9
120	28	38.6	120	10	16.8	103	0	0.0
98	43	61.9	66	15	22.5	27	6	10.8
98	34	56.0	86	20	39.8	64	0	0.0

* Arterialised venous insulin concentrations (μU/ml)

** Arterialised venous - mixed venous insulin differences (μU/ml)

§ Forearm insulin uptake (μU/100 ml forearm/min)

TABLE 5

Increment in glucose entry into general circulation following 100 g oral glucose load in normal subjects

Time	Total peripheral glucose uptake	Change in glucose pool	Increment in glucose entry
min	g	g	g
0 - 15	1.09	+ 7.98	9.07
15 - 30	2.86	+ 7.98	10.84
30 - 45	3.95	- 0.21	3.74
45 - 60	4.62	- 2.10	2.52
60 - 75	4.72	- 2.52	2.20
75 - 90	4.41	- 1.89	2.52
90 - 105	4.03	- 1.89	2.14
105 - 120	3.74	- 1.05	2.69
120 - 135	3.47	- 1.26	2.21
135 - 150	3.30	0.0	3.30
150 - 165	3.01	- 1.26	1.75
165 - 180	2.47	- 0.42	2.05
			45.03

TABLE 6

Comparison of incremental areas under response curves following 100 g oral glucose load before and after carbohydrate restriction in 13 normal subjects

	Minutes					
	0 - 60			60 -- 120		
	Before	After	P	Before	After	P
Glucose* units	61 ± 4**	81 ± 4	< .001	48 ± 5	113 ± 9	< .001
Insulin units	51 ± 5	37 ± 4	< .05	55 ± 7	78 ± 7	< .005
Glucose uptake units	.446 ± .048	.376 ± .035	N.S.	.585 ± .049	.576 ± .066	N.S.

* Refers to arterialised venous glucose curve
** Standard error of mean

TABLE 6 Cont.

Comparison of incremental areas under response curves following 100 g oral glucose load before and after carbohydrate restriction in 13 normal subjects

	Minutes					
	120 - 180			0 - 180		
	Before	After	P	Before	After	P
Glucose* units	23 ± 4**	85 ± 9	< .001	132 ± 10	279 ± 18	<.005
Insulin units	31 ± 4	62 ± 7	< .001	137 ± 15	177 ± 12	<.005
Glucose uptake units	.390 ± .033	.538 ± .064	<.01	1.421 ± .098	1.490 ± .147	N.S.

* Refers to arterialised venous glucose curve
** Standard error of mean

TABLE 7

Responses of G.T. and G.M. following 100 g oral glucose load before and after carbohydrate restriction

Subject			Minutes												
			0	15	30	45	60	75	90	105	120	135	150	165	180
G.T.															
Glucose mg/100 ml	AV*	Before	83	118	172	175	151	142	142	13'	125	112	114	124	92
		After	69	128	209	231	250	230	216	201	200	200	175	192	170
	MV**	Before	83	102	148	146	120	110	12+	117	106	92	95	108	86
		After	68	111	172	175	185	171	163	165	149	143	138	132	136
Glucose uptake mg/100 ml forearm/min		Before	.000	.280	.312	.473	.605	.605	.398	.398	.519	.390	.543	.541	.250
		After	.008	.163	.489	.705	.858	.855	.800	.522	.924	.826	.670	1.160	.595
Insulin µU/ml		Before	8	28	61	60	38	38	41	41	38	36	35	44	18
		After	7	37	58	68	110	74	105	72	100	92	81	74	54
G.M.															
Glucose mg/100 ml	AV	Before	89	105	161	170	170	152	134	122	100	105	105	115	113
		After	68	121	168	198	178	143	153	117	125	138	145	136	119
	MV	Before	85	91	119	124	116	104	101	86	73	84	88	92	94
		After	69	102	102	120	121	107	109	86	99	104	106	104	102
Glucose uptake mg/100 ml forearm/min		Before	.066	.189	.618	.883	1.108	1.013	.677	.713	.554	.404	.336	.631	.572
		After	.000	.200	.694	.678	.638	.446	.629	.404	.356	.486	.582	.617	.296
Insulin µU/ml		Before	9	14	53	62	70	74	64	58	33	44	41	48	42
		After	6	49	62	90	100	65	68	37	41	55	58	55	50

* Arterialised Venous

** Mixed Venous

TABLE 8

Response to regional intra-arterial insulin infusion before and after carbohydrate restriction in normal subjects

Subject		Minutes								
		0	10	20	30	40	50	60	70	80
D.T.										
Glucose * mg/100 ml	Before	84	85	83	82	82	78	77	80	82
	After	65	63	63	63	63	63	63	64	66
Glucose uptake mg/100 ml forearm/min	Before	.085	.232	.499	.474	.536	.249	.172	.096	.057
	After	.018	.021	.149	.313	.284	.286	.130	.092	.086
Insulin (MV)** µU/ml	Before	9	70	70	74	12	8	9	6	6
	After	<6	45	74	48	8	6	7	6	7
R.H.										
Glucose * mg/100 ml	Before	67	64	66	66	65	68	70	73	75
	After	57	55	53	51	53	55	57	58	59
Glucose uptake mg/100 ml forearm/min	Before	.038	.026	.182	.207	.228	.230	.275	.199	.228
	After	.000	.024	.058	.076	.055	.083	.028	.015	.036
Insulin (MV)** µU/ml	Before	7	98	108	112	21	14	15	14	13
	After	7	76	72	76	11	9	7	6	6

* Arterialised venous

** Mixed venous

TABLE 9

Acetoacetate uptake and β-hydroxybutyrate release by forearm tissues following 100 g oral glucose load in seven normal subjects after carbohydrate restriction

	Minutes						
	0	30	60	90	120	150	180
Acetoacetate uptake μmol/100 ml forearm/min	.619 ± .121*	.463 ± .073	.514 ± .120	.288 ± .114	.195 ± .109	.167 ± .095	.167 ± .064
β-hydroxybutyrate release μmol/100 ml forearm/min	.50 ± .18	1.14 ± .50	.81 ± .50	.77 ± .36	.92 ± .39	.52 ± .21	.24 ± .10

* Standard error of mean

TABLE 10

Insulin concentrations (μU/ml) following intravenous injection of exogenous insulin before and after carbohydrate restriction

Time	C.H.		A.Y.		M.B.	
min	Before	After	Before	After	Before	After
0	14	5	14	5	0	6
3	580	840	-	980	420	630
6	320	610	-	710	300	420
9	245	490	240	570	160	146
12	105	270	140	430	82	146
15	95	125	140	150	61	107
18	74	100	82	125	51	76
21	62	79	60	96	31	65
24	41	67	58	85	34	52
27	40	55	55	74	24	42
30	38	40	40	55	23	33
33	34	39	39	54	18	30
36	28	38	31	45	17	27
39	28	35	31	39	16	24
42	24	29	30	28	16	21
45	19	24	25	29	-	20

TABLE 11

Increment in glucose entry into general circulation following 100 g oral glucose load in normal subjects before and after carbohydrate restriction

Time	Total peripheral glucose uptake (g)		Change in glucose pool (g)		Increment in glucose entry (g)	
min	Before	After	Before	After	Before	After
0 - 15	1.29	1.02	+ 8.60	+ 8.81	9.89	9.83
15 - 30	3.55	2.80	+ 8.83	+10.50	12.38	13.30
30 - 45	4.74	4.21	- 1.05	+ 7.55	3.69	11.76
45 - 60	5.36	4.79	- 2.52	+ 0.63	2.84.	5.42
60 - 75	5.66	4.92	- 2.10	- 2.31	3.56	2.61
75 - 90	5.15	4.73	- 1.89	- 1.05	3.26	3.68
90 - 105	4.66	4.61	- 1.47	- 1.47	3.19	3.14
105 - 120	4.26	5.05	- 1.47	- 1.47	2.79	3.58
120 - 135	3.65	4.93	- 2.73	- 1.68	0.92	3.25
135 - 150	3.46	4.60	+ 0.21	- 1.26	3.67	3.34
150 - 165	3.34	4.55	- 0.63	- 1.86	2.71	2.69
165 - 180	2.67	4.01	- 0.84	- 2.31	1.83	1.70

TABLE 12

Comparison of incremental areas under response curves following 100 g oral glucose load before and after carbohydrate restriction in five diabetics

	Minutes					
	0 - 60			60 - 120		
	Before	After	P	Before	After	P
Glucose* units	84 ± 9**	82 ± 11	N.S.	182 ± 15	198 ± 15	N.S.
Insulin units	7.3 ± 2.5	10.5 ± 3.7	N.S.	17.4 ± 6.1	19.4 ± 4.2	N.S.
Glucose uptake units	.383 ± .036	.341 ± .089	N.S.	.398 ± .062	.522 ± .119	N.S.
$\frac{\Delta \dot{I}nsulin}{\Delta Glucose}$ (x 100)	92 ± 39	131 ± 39	N.S.	103 ± 43	101 ± 22	N.S.
$\frac{\Delta \text{ Glucose uptake}}{\Delta Insulin}$ (x 100)	134 ± 79	50 ± 18	N.S.	41 ± 16	29 ± 7	N.S.

* Refers to arterialised venous glucose curve
** Standard error of mean
‡ Refers to incremental area under response curve

TABLE 12 Cont.

Comparison of incremental areas under response curves following 100 g oral glucose load before and after carbohydrate restriction in five diabetics

	Minutes					
	120 - 180			0 - 180		
	Before	After	P	Before	After	P
Glucose* units	172 ± 15**	240 ± 17	.02	458 ± 37	520 ± 40	N.S.
Insulin units	17.7 ± 6.1	27.0 ± 4.3	N.S.	42.1 ± 14.2	57.2 ± 11.7	N.S.
Glucose uptake units	.128 ± .055	.400 ± .128	.05	.909 ± .125	1.263 ± .334	N.S.
$\frac{\Delta \text{Insulin}}{\Delta \text{Glucose}}$ (x 100)	111 ± 44	118 ± 25	N.S.	103 ± 41	114 ± 25	N.S.
$\frac{\Delta \text{Glucose uptake}}{\Delta \text{Insulin}}$ (x 100)	11 ± 5	15 ± 4	N.S.	35 ± 11	23 ± 5	N.S.

* Refers to arterialised venous glucose curve
** Standard error of mean
‡ Refers to increnental area under response curve

TABLE 13

Response following 100 g oral glucose load in diabetic (K.P.) and age-matched nondiabetic (J.B.)

		Minutes												
		0	15	30	45	60	75	90	105	120	135	150	165	180
Glucose* mg/100 ml	K.P.	239	262	314	344	367	385	401	392	376	378	391	375	358
	J.B.	96	137	194	191	153	135	127	132	128	121	119	109	113
Glucose uptake mg/100 ml forearm/min	K.P.	.000	.180	.585	.428	.464	.476	.443	.234	.073	.095	.174	.000	.000
	J.B.	.048	.179	.453	.519	.262	.189	.246	.202	.245	.230	.267	.183	.175
Insulin μU/ml	K.P.	6	7	7	6	8	8	12	11	9	12	14	15	17
	J.B.	7	21	58	85	56	34	35	45	35	35	37	26	29

Arterialised venous plasma

TABLE 14

Response following 100 g oral glucose load in diabetic (T.R.) and age-matched nondiabetic (T.P.)

		Minutes												
		0	15	30	45	60	75	90	105	120	135	150	165	180
Glucose* mg/100 ml	T.R.	228	256	301	350	393	420	444	437	446	448	430	406	378
	T.P.	86	120	168	188	180	184	173	155	142	131	141	133	130
Glucose uptake mg/100 ml forearm/min	T.R.	.106	.332	.695	.752	.945	.865	.765	.596	.539	.471	.350	.000	.000
	T.P.	.042	.210	.498	.524	.904	1.070	.793	.796	.615	.591	.710	.529	.414
Insulin µU/ml	T.R.	8	9	14	15	17	24	19	17	18	14	17	13	13
	T.P.	14	30	68	60	54	98	102	94	94	65	72	64	65

* Arterialised venous plasma

TABLE 15

Response following 100 g oral glucose load in diabetic (J.W.) and age-matched nondiabetic (A.P.)

		Minutes												
		0	15	30	45	60	75	90	105	120	135	150	165	180
Glucose* mg/100 ml	J.W.	91	124	180	231	218	218	219	207	202	193	149	141	141
	A.P.	84	135	163	108	99	104	118	134	119	99	122	119	107
Glucose uptake mg/100 ml forearm/min	J.W.	.014	.211	.500	.955	.584	.590	.734	1.180	1.005	1.028	.035	.835	.456
	A.P.	.036	.350	.718	.445	.475	.542	.570	.476	.447	.357	.438	.488	.348
Insulin μU/ml	J.W.	21	33	79	88	150	69	125	175	150	200	165	135	135
	A.P.	5	47	130	33	20	20	24	38	29	15	34	30	20

* Arterialised venous plasma

TABLE 16

Response following 100 g oral glucose load in normal subject (D.S.)

		Minutes								
		0	15	30	45	60	75	90	105	120
Test a	Glucose AV* mg/100 ml	84	129	180	193	201	183	159	126	111
	MV**	83	100	136	162	158	148	124	102	99
Test b	Glucose AV mg/100 ml	85	129	160	151	128	114	108	108	108
	MV	85	95	117	90	79	71	68	68	72

* Arterialised venous
** Mixed venous

TABLE 17

Glucose concentrations (mg/100 ml) in arterialised venous (AV) and mixed venous (MV) plasma following 100 g oral glucose load in a normal subject (C.B.).

	Minutes												
	0	15	30	45	60	75	90	105	120	135	150	165	180
AV	81	118	124	130	135	136	130	132	120	118	124	116	110
MV	76	98	76	82	88	96	91	88	84	75	81	77	84

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