THE MEASUREMENT AND MODELLING OF BLOOD GLUCOSE DYNAMICS IN MAN

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ABSTRACT

The aim of the work presented in this thesis is to establish a mathematical model for use in the study of blood glucose dynamics and their disorders in man.

A large number of models have been developed that represent various aspects of blood glucose dynamics and of these the majority have been attempts to provide a mathematical description of glucose tolerance test curves. Most of these models have no immediate clinical application and have also failed to provide any real insight into the system that controls the flow of glucose into and from the blood glucose compartment.

To do this a mathematical model must be formulated that approximates to an isomorphic description of the glucose regulatory system. Such a model must be based on sound biochemical and physiological knowledge. However, the complexity of the glucose system is such that complete simulation is not possible.

In this study, a model has been developed and is described that places particular emphasis upon the role of the liver in controlling blood glucose dynamics. Compartments are provided for glucose, glucose-6-phosphate, and glycogen, which are the dominant metabolic substrates. Mass balance equations have been written in terms of the enzymatic reactions that are involved in glucose transport and substrate kinetics. Insulin and glucagon hormonal controllers have also been incorporated.

The model has been tested by inputs representing intravenous glucose infusion, intravenous glucose, insulin and glucagon injection.

The model appears to simulate in general terms experimental data, and has yielded information about both the system structure and the enzyme dynamics involved. Of particular importance is the evidence that fine control of the glucose system is an intrinsic function of the enzyme systems and that only coarse control of the system is provided by the hormonal environment.

Little work has been done in this field, but the present model is unlike any previous model in that the inherent non-linearities of the metabolic system are developed from known enzymological data. The approach illustrates the possible value of using kinetic data obtained from *in vitro* experiments in predicting physiological changes within the *in vivo* system.

It is considered that mathematical models of this type can provide the biomedical scientist with insight into the functioning of metabolic systems and highlight areas of weak knowledge.

CONTENTS

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I should like particularly to thank Professor Victor Wynn of St. Mary's Hospital Medical School who stimulated my interest in the metabolic control of glucose homeostasis and in whose department this work was conceived and the greater part carried out.

However the work would not have come to a fruitful conclusion without the encouragement of Professor D.N. Baron of the Royal Free Hospital School of Medicine in whose department the work was completed, and whom I wish to thank.

I am indebted to Mr. Ewart R. Carson of the Department of Systems Science, the City University, for so patiently tutoring me in the methods of systems science and discussing their application to metabolic problems.

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CONVENTIONS

As far as has been possible the abbreviations, conventions and symbols used in this thesis are those recommended by the Editorial Board of the Biochemical Journal in their Policy of the Journal and Instructions to Authors (Revised Version 1973).

References are given in the form used in the Biochemical Journal but with both the first and last page number being cited, together with the full title of the paper.

At the first mention of an enzyme there is given the number assigned to it in Enzyme Nomenclature: Recommendations (1972) of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry on the Nomenclature and Classification of Enzymes together with their Units and the Symbols of Enzyme Kinetics (Amsterdam : Elsevier Scientific Publishing Co., 1973).

Enzyme numbers are given in the accepted form, EC 1.2.3.4.

' ... many biologists, like scientists in other fields, are too concerned with the discovery of experimental data, without being prepared to provide the necessary integrated theoretical background. It is one of the sad features of modern scientific development that theory is too often divorced from practice, an attitude that reflects against the belief, held by so many scientists, that science can develop by the accumulation of facts alone.'

(George, 1965)

'In a growing research discipline, inquiry is not directed towards re-arranging old facts and explanations .... but rather to the discovery of new patterns of explanation.'

(Hanson, 1965)

CHAPTER 1

INTRODUCTION

I. The nature of the problem

It has been known for more than fifty years that diabetes is characteristically associated with a decrease in glucose tolerance (Janney and Isaacson 1918). An increase in the fasting blood glucose is not invariable, and the most reliable laboratory procedure is the glucose tolerance test, which since its popularisation by Hamman and Hirschmann (1917) has become the most important test for the diagnosis of diabetes mellitus. Most practical for routine use and thus most commonly performed is the oral test. The intravenous test eliminates the problem of equivocal results due to defective alimentary absorption and allows calculation of the glucose "utilisation rate". Nevertheless our knowledge of blood glucose dynamics is still mainly descriptive rather than explanatory. The distribution and disposition of glucose load and the maintenance of glucose homeostasis in the body are the results of a complex set of physiological processes and biochemical interactions. The readily observable dynamic events are the plasma, or blood, glucose concentration and its changes, and to a lesser extent the amount of glucose excreted in the urine. Consequently, the glucose tolerance test, in its oral or intravenous form, remains the most commonly used tool for the study of glucose metabolism in man, both in clinical practice and in research. Recognising the inadequacies of these tests as so often performed, there have been numerous attempts to introduce

standardised methods of performance and of interpretation (Joplin and Wright 1968). Nevertheless, the relationship between the conformation of the glucose tolerance curve and the mode of disposing of the glucose load is still poorly understood. This is probably due to the fact that this curve is the result of several metabolic processes and as such is unlikely to provide information about the dynamics involved.

Some insight into the mechanisms which control blood glucose levels may be obtained by introducing theoretical models. Several authors have used such an approach in an attempt to describe plasma glucose dynamics after the steady state has been disturbed by oral or intravenously injected glucose.

Using information obtained from such experimental procedures, a formal compartmental model can be postulated and its parameters chosen according to statistical criteria to produce a "best" fit to the data. The weakness of this approach is that it is essentially an optimisation procedure whereby a set of numerical values are found that give best fit of a predetermined model to experimental data. Its use has been critically reviewed by Atkins (1971).

Such descriptions of the blood glucose regulating system do not give, furthermore, information about actual rates of utilisation and release of glucose from and into the blood, although some consider in a theoretical way the net changes of the amount of circulating glucose, which are due to differences between these rates. Yet it is by varying these rates that control mechanisms are able to maintain the circulating blood glucose within a normal optimal range in spite of varying external factors such as fasting, or the ingestion or injection of glucose. Changes in the plasma or blood glucose concentration represent the integration over a time period of the differences between glucose utilisation and glucose release. This glucose concentration

is in itself an insensitive indicator of these values. But if information about glucose utilisation or release, whether in the steady-state or following glucose loading, is required, these rates must be calculated.

In the final analysis the mechanisms by which all biological processes take place are chemical in nature and must be explained ultimately in the transformation of one chemical species into another, with each transformation being catalysed by an enzyme. Therefore, it should be possible to describe the disposition of glucose in the body in dynamic terms using the linked reactions of the system for glucose metabolism. This system is an obvious candidate for the study of a problem *in vivo*, the sub-systems of which have been intensively studied in the varying tissues *in vitro*.

The individual enzyme reactions are known, the substrates and coenzymes have been identified, the enzymes isolated and their properties studied. There is also significant information, usually obtained *in vitro*, as to how these reactions are controlled at the enzyme level. What is required is a model built to describe the system, then it should be theoretically possible to describe the individual physiological processes and biochemical interactions in operational terms, and, if sufficient data are available, or obtainable, to predict those events that can be observed. Such dynamics are complex and require a large number of parameters: often far more parameters are needed than can be justified on the basis of a statistical fit to plasma and excretion data. Generally, however, most of the parameters can be predicted within reasonable limits because much literature exists which reports the quantitative behaviour of physiological systems.

In fact, one of the primary experimental techniques for inferring

the behaviour of the processes within the body is to observe how the processes deal with an exogenous dose of glucose. The processes by which glucose is distributed within the body are physiological and physicochemical. Much is known about these physiological processes, thus it is possible that such an approach will be rewarding because

a) there is much information readily available in the literature;

b) extrapolations may be attempted with some confidence if the mechanisms are understood;

c) behaviour of the system may be predicted a priori;

d) compartments correspond to anatomical spaces so that biochemical interactions can be incorporated.

In fact, of recent years there have been attempts to bring system theory into the quantitative study of physiological regulatory processes.

The general philosophy of this approach, which has been called systems physiology, has been outlined by Mommaerts et al (1968). Briefly its objective is to determine the interconnections and couplings, within a system, and then model and dynamically test the system. The goal is a mathematical model and/or computer model which couples the dynamics of each subsystem and results in a faithful dynamic model of the overall system. The achievement of this goal provides more than a simulation of the system because it also provides information concerning the functional properties of the system. Information of this sort has immense practical implications for the diagnosis and treatment of metabolic disorders.

II. The scope of the study

The aim of the work presented in this thesis is, using the systems approach, to establish a mathematical model for use in the study of blood glucose dynamics and investigations of its disorders in man.

This is essentially a mechanistic model based on the unit processes of the system, that is the enzyme reactions. Little work has been done in this field. Models of the glycolytic pathways have been developed by the group associated with Chance and Garfinkel (see for review, Garfinkel 1969), but such an approach has not been attempted for the blood glucose regulatory system.

The present study considers the glucose regulatory system in the context of a mathematical model based on the complex reaction system of the glucose metabolic network and involving the kinetics of the various enzyme and transport reactions. Thus in this model unlike any previous model the inherent non-linearities of the system are developed from known enzymological data. This approach illustrates the value of using kinetic data obtained <u>in vitro</u> in predicting physiological changes within the <u>in vivo</u> system.

Firstly, the whole concept of blood glucose dynamics is put into its historical perspective, with particular consideration being made of the role of the liver, and earlier attempts of modelling the system. Then, after a detailed exposition of the theoretical considerations involved, the development of the mathematical model is described. Evaluation of the model and the system by computer simulation is considered. The results section includes an appraisal of physiological data obtained from the procedures described, and finally the acceptability of the method to clinical problems is discussed.

CHAPTER 2

A HISTORICAL ACCOUNT OF THE STUDY OF BLOOD GLUCOSE DYNAMICS

Modern physiological concepts of glucose metabolism and their relation to blood glucose dynamics have their origin in the studies of Claude Bernard. In 1848, Bernard demonstrated the existence of sugar in the blood of animals in the absence of prior carbohydrate feeding. In two healthy dogs sugar was present in blood taken from the heart after death. One dog had been fed on a meal which contained no starch; the other animal had received no food for two days. In subsequent experiments to determine the origin of the sugar Bernard placed ligatures at various places in the portal venous system immediately after death. No sugar was found 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 concluded that the sugar originated in the liver. At that time he believed that the sugar from the liver was the same as that of diabetes but that both differed from grape sugar; subsequently (1877) he acknowledged their identity.

One of the most important results of the finding of glucose in the blood of fasting animals was the discovery of glycogen. By Bernard's own admission this was fortuitous. Normally duplicate estimations of the sugar content of liver tissue were performed simultaneously but on one occasion, when pressed for time, one estimation was made immediately after the death of the animal and the other was delayed until the following day. The second estimation gave a much higher result than the first. On investigating the discrepancy he found that the time

factor was of great importance in determining the amount of sugar present in the specimen. Only a small amount of glucose might be present soon after death but at the end of two hours a large amount of sugar had appeared. He perfused a still warm liver with cold water and freed the tissue of sugar. Subsequently large amounts of sugar appeared, proving that the sugar was formed from material in the liver tissue itself (Bernard 1855). Bernard (1857) went on to isolate the sugar-forming substance, which he named 'glycogen', and described many of its chemical and physical properties. Sanson (1857) found that muscle tissue also contained a considerable amount of glycogen.

Young (1937) pointed out that Bernard's early theories on the fate of the sugar liberated by the liver were erroneous. Bernard believed that the sugar 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 1855,b). For many years Bernard also insisted that the portal blood of a dog fed only on meat did not contain sugar. Several investigators disagreed strongly with him on this point. Figuier (1855) found sugar in the portal vein of a dog two and a half hours after ingestion of meat but found no glucose in the portal blood of an animal which had been starved of food. Chaveau (1856) conducted a careful study of the concentration of sugar in a variety of vessels; he found it in the portal vein and in the blood draining the small intestine in animals fasted for forty-eight hours. 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 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 sugar present in arterial blood than in venous blood.

Pavy (1860) also found sugar in the portal blood in amounts similar

to those found under normal circumstances in the heart and other vessels of the body. When blood was taken from the heart during life only a trace of sugar was detected but large amounts were found shortly after the death of the animal. Pavy also showed that respiratory obstruction (Bernard 1855) or struggling (Pavy 1860) caused an unnatural increase of the sugar in the circulation.

Studies on the relative amounts of sugar in different vessels set the scene for the investigation of the fate of ingested glucose. This problem was the starting point of Bernard's investigations. His "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" (Bernard 1853).

Bernard's own views on the fate of absorbed sugar were contradictory. In 1877 he 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." This suggests that Bernard believed that the liver blocked the passage of sugar through it; a view was apparently confirmed by his observation that if a given quantity of glucose was injected into the jugular vein of a dog it passed more readily into the urine than if the same quantity of glucose was injected into a branch of the portal vein. Bernard further showed that feeding of potatoes to a dog with an obstruction of the portal vein resulted in a definite glycosuria and quoted as clinical confirmation the presence of diabetes in a subject with portal vein obstruction and alimentary glycosuria in cirrhotics with portal vein obliteration.

However in the same book 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 organs, in all the tissues." He thus recognised that other tissues could utilize glucose; indeed some of his researches led him to question the role of dietary sugar in the production of hepatic glycogen. In 1858 (Bernard 1877) he performed experiments indicating that the liver of a dog fed on starch contained less glycogen than that of a dog fed on fibrin; other experiments led him to believe that glucose, sugar and starch were not able to give rise to liver glycogen in fasting animals to which these foods alone were given. He concluded that cane sugar, and probably the other carbohydrates, were 'nutritive stimulators' of glycogen formation but that they were not directly converted to glycogen. Bernard wrote that nitrogenous matter was the chief source of the glycogen manufactured by the liver.

Pavy had a different concept of the fate of ingested glucose and the role of the liver. He found in 1858 that the total amount of glycogen in the liver of sugar fed animals was much greater than that to be found in the liver of animals receiving other types of food. He therefore considered that liver glycogen might be formed from the sugar in food. In 1862 he found that the blood flowing from the liver of an animal 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. This indicated that the liver was absorbing the ingested sugar (see Young 1937).

Pavy (1894) 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, in which there was a high blood sugar, led Pavy to suggest that glucose was toxic in the body and

that the liver protected the organism by converting the sugar to glycogen.

The main problem in all the studies performed up to this time was the lack of a reliable method for the measurement of blood sugar in small amounts of blood. Serial estimates could not be performed and it was impossible to follow with any accuracy changes in the concentration of blood glucose in response to glucose loading.

The prime stimulus for research into carbohydrate metabolism in man was the prevalence and serious nature of diabetes mellitus. Apart from its clinical characteristics the main diagnostic feature of the disease was glycosuria. This was usually detected by testing for reducing substances and the presence of glucose itself was occasionally confirmed by the demonstration of fermentation, dextrorotation of the plane of polarised light and osazone formation. Normal human beings and animals were found to have small amounts of reducing substance in the urine. This was assumed to be an indication of the presence of glucose or another sugar in the urine.

The recognition that significant glycosuria could be caused, under certain circumstances, by the administration of sugars to normal humans and animals stimulated interest in the quantitative relationship between glycosuria and the diabetic state. The concept of 'carbohydrate tolerance' originated at this time. According to Folin and Berglund (1922) this term originally represented 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. A different meaning was used after Hofmeister (1888-9) introduced the term 'limit of sugar assimilation' to indicate the amount of sugar which could be taken in one dose to 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 laevulose. 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."

Folin and Berglund (1922) pointed out that the original concept of alimentary glycosuria (or 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 nature of this assumption was pointed out by Linossier and Rogue (1895) who "..... expressed the fact that the more sugar that is taken the more is retained and utilized by non-diabetic individuals." However the inadequacy of this concept was demonstrated by Pavy and Godden (1911) 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.

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. However the blood sugar level was not the only factor determining glycosuria. 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 the other factors governing glycosuria, may produce effects in either direction, either towards causing glycosuria or towards preventing it."

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.

The need for a reasonably accurate micro-method of measuring blood sugar was first met by Bang (1913) with his iodide-reduction method. Numerous other workers subsequently presented new methods of increasing sensitivity and accuracy (see Henry 1960 for a review of methods). So gradually the assessment of carbohydrate tolerance was determined not by the presence or absence of glycosuria but by the height of the blood sugar rise following the administration of oral glucose.

Jacobsen (1913) used Bang's method to show that after ingestion of 100g glucose an appreciable rise in the blood sugar occurred, in a normal subject, within five minutes: the maximum level was usually attained in about thirty minutes, and a return to pre-ingestion levels occurred within an hour and three quarters. He tested several diabetics with 50g of bread and showed that the actual rise of the blood sugar was not great but that the fall was slow. The paper of Hamman and Hirschman (1917) really popularised the tolerance test and subsequently the changes in blood level after oral glucose administration were widely used by clinicians, and in experimental studies in animals and man as an index of the efficiency of blood sugar regulatory mechanisms. The early work was reviewed by Maclean and de Wesselow (1921) and Hale-

White and Payne (1926) and more critical reviews have been presented by Baird and Duncan (1959), Roeckel (1971) and De La Huerga and Sherrick (1971).

A variety of tests have been employed using varying amounts of glucose in an attempt to make interpretation of the test more reliable. In this country the British Diabetic Association recommend that 50g of glucose be administered to a subject who has been fasting overnight, whilst in the United States it is the usual practice to administer 100g. Intermediate doses such as 75g, or doses proportional to body weight, such as one or 1.75g per kg body weight have been used. In 1969 the American Diabetic Association recommended a dose of 40g per square metre of body surface. The blood glucose is determined usually on venous blood obtained before glucose loading and then at timed intervals after glucose ingestion. The intervals are usually every 30 minutes for a period of 2-3 hours. Urine is sometimes collected at intervals similar to a crude method of assessment of the renal threshold for glucose. Other difficulties have arisen in attempts to standardise the test, for accurate comparisons are almost impossible as different glucose loads have been used, and capillary, venous blood or plasma analysed by numerous analytical methods.

Furthermore, it has become increasingly apparent that intolerance of glucose is not an exclusive feature of clinical diabetes mellitus, but may be the result of very many physiological and pathological changes.

Little work has been done on the physiological mechanisms responsible for the shape of the oral glucose tolerance test 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. Bailey (1919) 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 oral glucose and in three subjects given glucose directly into the duodenum. They also showed that the blood sugar could return to normal while glucose was still present in the stomach and that it could start falling while a considerable amount of glucose was present in the stomach.

The role of the liver in handling glucose absorbed from the intestine has been emphasised since the early work of Bernard and Pavy. The importance of the peripheral tissues was not fully appreciated until large arterio-venous glucose differences were demonstrated after oral glucose loading (Foster 1923). Sömögyi (1948) investigated this problem extensively; he pointed out that arterio-venous differences were very small in pancreatectomized animals but that they increased considerably after the injection of insulin. An increase in arterio-venous differences occurred in normal subjects during alimentary hyperglycaemia; this was attributed to an increased supply of insulin from the islets. Sömögyi emphasised the role of the extrahepatic tissues and suggested that during an alimentary hyper-glycaemia the muscles remove more glucose from the blood than does the liver.

Soskin and his colleagues (1934) questioned whether augmented insulin secretion following oral glucose was responsible for the shape of a normal glucose tolerance curve. They infused insulin at a constant rate into pancreatectomized dogs and by this means kept the blood sugar constant. They found that injection of glucose into these animals resulted in normal 'glucose tolerance curves'. It appeared

that provided sufficient insulin was present to maintain a constant blood sugar level, no additional secretion was necessary for adequate regulation. When normal dogs were hepatectomized, however, and a constant amount of glucose given to maintain a normal constant blood sugar level, the administration of glucose led to markedly 'diabetic' curves. This occurred in the presence of a normal pancreas. These findings suggested to Soskin and co-workers that the pancreas was not essential to the regulating mechanisms responsible for the normal glucose tolerance curve while the presence of the liver was essential. However the importance of the liver may not have been due to its capacity for glucose uptake as Lang, Goldstein and Levine (1954) demonstrated that the presence of the liver was necessary for efficient utilisation by the peripheral tissues.

Most of these studies were done before methods were available for the measurement of insulin in blood. In 1950(a) Bornstein demonstrated that following subcutaneous injection of insulin in alloxan-diabetic, hypophysectomized, adrenalectomized rats the fall of blood sugar correlated well with the amount of insulin administered. He used this preparation to assay 'insulin' in human plasma (Bornstein 1950b). Following the administration of 50g of glucose orally the plasma 'insulin' concentration increased as the blood sugar rose but its peak did not reach a maximum until two and a half hours after the ingestion of glucose - well after the peak of the glucose curve. This effect of oral glucose on the plasma insulin level was subsequently confirmed by biological assay of 'insulin activity' on the rat diaphragm (Vallance-Owen and Hurlock 1954) and on the tissue of the rat epididymal fat pad (Samaan, Fraser and Dempster 1963).

These methods of insulin assay depended on the measurement of biological effects which could have been affected by inhibitors of

insulin, by substances having the same action, or by factors potentiating the response to insulin itself. In 1959 Yalow and Berson introduced a radioimmunoassay method for plasma insulin of great sensitivity. These workers confirmed a finding which had been obtained with biological assays that insulin levels rise sharply after oral glucose.

The oral glucose tolerance test has retained its popularity. The vagaries of gastric emptying and intestinal absorption, however, led many workers to advocate the use of intravenous administration of glucose for assessment of the capacity of the body to assimilate glucose. Intravenous sugar was used when glycosuria was the only method of assessing tolerance but was found at that time to be unsatisfactory as tolerance depended less on the dose of glucose which was given than on the rate of administration. When repeated blood sugar determinations became possible the response of the blood sugar level to intravenous glucose administration became a practical method of assessing glucose tolerance. But, as with the oral test little experimental work has been done on the physiological significance of the intravenous tolerance test. Most authors have agreed that following the intravenous injection of glucose there is an immediate rise in the blood level; this initially falls rapidly as the result of diffusion throughout the extracellular fluid together with the loss of a small amount into the urine. When this rapid equilibration has occurred the rate of disappearance from the blood is thought to reflect the net metabolic removal of glucose by the tissues.

Tunbridge and Allibone (1940) reviewed early studies and pointed out the confusion which arose owing to the varying strengths and amounts of glucose solution used and the varying time taken for injection. In 1913 Thamhauser and Pfitzer injected 35g of glucose over a period of fifteen minutes and found that in normal subjects and those with mild

diabetes the blood sugar fell rapidly to pre-injection values while in subjects with liver disease and more severe diabetes it took a much longer time before the excess of sugar was eliminated from the blood. Jörgensen and Plum (1923) gave 20g of glucose by rapid intravenous injection and found in normal subjects a rapid fall in blood sugar concentration which dropped below pre-injection values. In diabetics they also found a very rapid initial fall but the curves later became progressively less steep causing the area under the curve to increase.

The intravenous glucose tolerance test using a single bolus load has been widely used for many years. The rapid injection method of assessing intravenous tolerance is the one that has been most generally accepted. Even so there is little uniformity of procedure, particularly with respect to the dose of glucose injected, and there has been considerable lack of agreement as to the best method of evaluating the results obtained. Jörgensen and Plum (1923) used the area of the curve and the time taken for the blood sugar level to return to pre-injection values. Crawford (1938) used the time taken to reach the value of 100 mg/100 ml, while Lozner and his colleagues (1941) used the two hour blood sugar value as their index.

The principal advantages of this test over oral glucose tolerance tests are that it eliminates the problem of variable intestinal absorption and gives a quantitative index of carbohydrate utilization by allowing calculation of the specific rate constant k from a glucose-time relationship; k has commonly been derived from the exponential equation originally introduced by Hamilton and Stein (1942):

$$C_G = C_o e^{-kt}$$

where C_G is the blood glucose concentration at any time t and C_o the concentration at time zero.

This equation would be justified if a logarithmic plot of glucose concentrations as a function of time were to produce a straight line. If the slope of the glucose curve is plotted against the corresponding glucose values a linear relationship with a positive intercept implies that a constant term is present in the equation (Figure 1). This constant is equal neither to zero nor to the fasting glucose concentration, and is the asymptotic value of the decay curve (C_{EQ}) (Greville 1943, Hlad, Elrick and Arai 1959). Thus the above equation can be rewritten:

$$C_G = (C_o - C_{EQ})e^{-kt} + C_{EQ}$$

The glucose plot fits closely to this equation up to 90 minutes after injection of the glucose. This expression is consistent with a constant rate of glucose input, reflected by the term C_{EQ}, and an exponential decay in the rate of dissimilation, characterised by the term $-k$.

However, Conard and his colleagues (1953) and Ikkos and Luft (1957) reverted to the simpler method of Hamilton and Stein and plotted graphically on semi-logarithmic paper the total blood glucose concentration against time. They called the slope of the straight line which best fitted their experimental data the 'glucose assimilation coefficient'. This method is probably the most widely used today for the assessment of intravenous glucose tolerance, a constant, k, being used to describe the rate of disappearance of glucose. k is calculated simply from the equation:

$$k = \frac{0.693}{t_{\frac{1}{2}}}$$

where $t_{\frac{1}{2}}$ is the time required for the level of glucose excess to be reduced by one half its value.

The derivation of this expression is as follows:

The half-life period ($t_{\frac{1}{2}}$) of a substance is the time taken for the

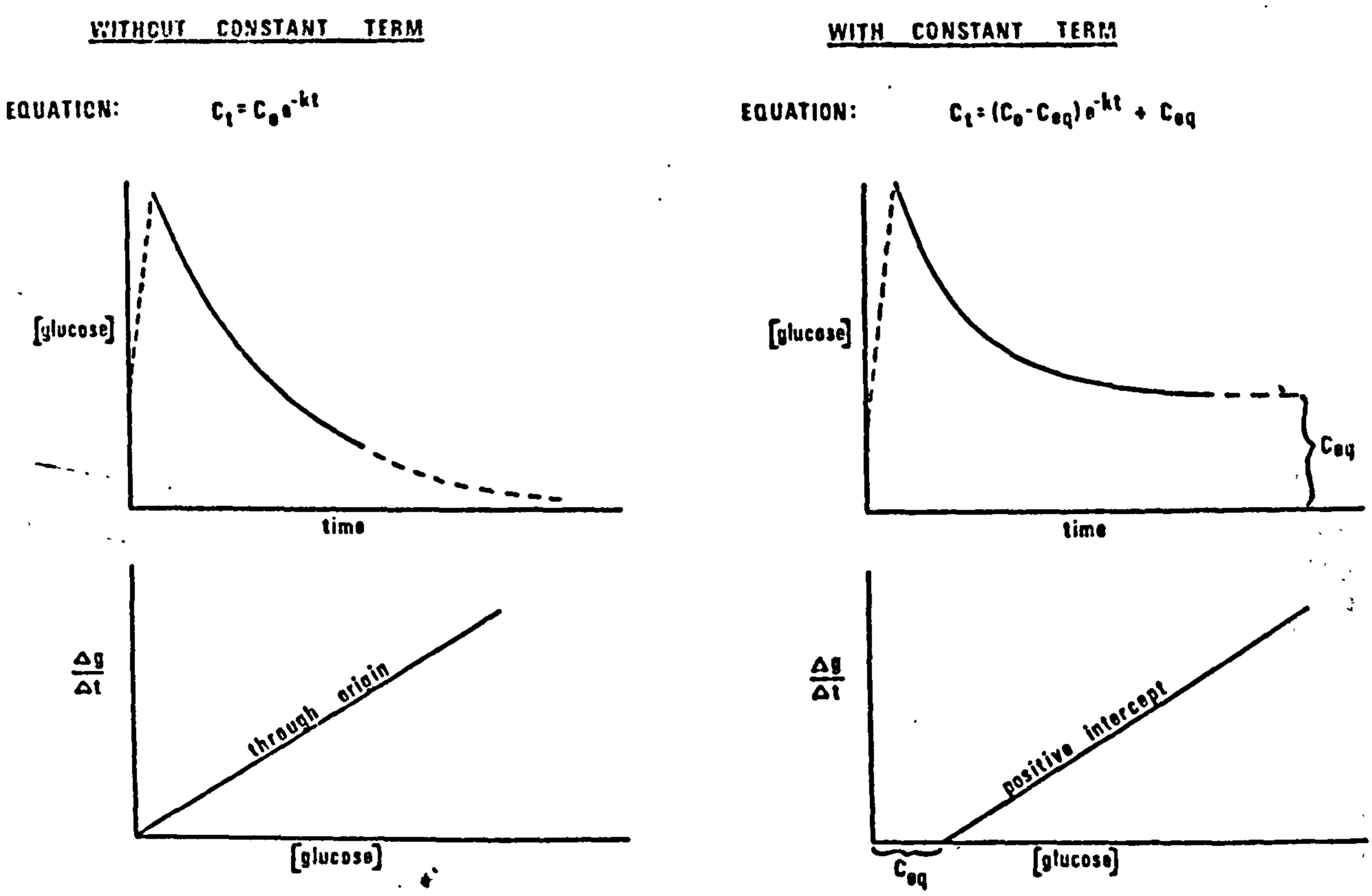


Figure 1

Form of glucose curves derived from equation with and without constant term.

concentration of the substance to decrease by one half.

For a first-order reaction the kinetic equation is

$$\log \frac{a}{a-x} = \frac{kt}{2.303}$$

substituting $t\frac{1}{2}$ for t and $\frac{1}{2}a$ for both x and (a-x) the relationship between the value to $t\frac{1}{2}$ and the initial concentration is

$$t\tfrac{1}{2} = \frac{2.303}{k} \log \frac{a}{\frac{1}{2}a}$$

$$= \frac{2.303 \log 2}{k}$$

$$= \frac{0.693}{k}$$

Transposing $k = \frac{0.693}{t\frac{1}{2}}$.

Cramp, Oakley and Johnson (1971) calculated the terms k and C_{EQ} from original intravenous test data using a curve fitting computer programme (Figure 2(a)). The principle of the procedure was that glucose values and their times of measurement were read into the computer (Figure 2(b)) and an initial set of values for C_{EQ} and k calculated (Figure 3(a)). These values were then improved upon by an iterative technique until the difference between successive approximations is very small.

Validation of goodness of fit of predicted curves was assessed on ten randomly selected intravenous tests in two ways; the mean percentage divergence over all points from 10-90 minutes for these tests is 2.2% (range 0.6 - 4.7), while the mean percentage of divergence at any point in time through the ten tests was 2.0% (range 1.1 - 3.5).

The Greville equation seems to be the best to describe blood glucose disappearance (Figure 3 (b)). Abnormality in glucose tolerance may be characterised by deviations from the normal of either or both k and C_{EQ} .

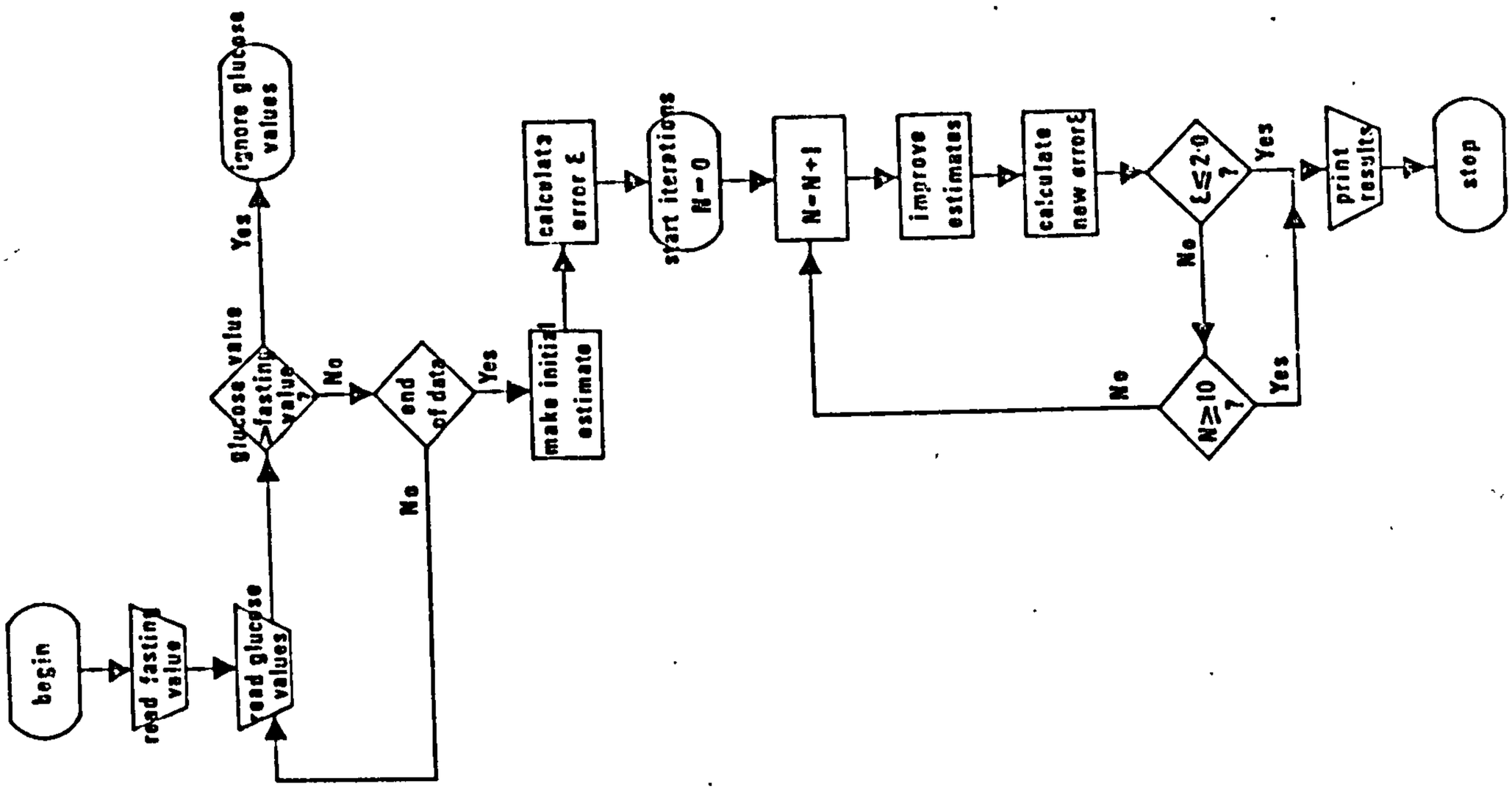


Figure 2(a)

Algorithm of curve fitting program.

SUB 202 A

*** RESULT AFTER 10 ITERATIONS , ESQUARED = 95.7991 ***

INITIAL	CORRECTED	DIFFERENCE
300	296.436	3.56408
225	232.429	-7.42932
186	185.909	9.05151E-02
155	152.099	2.90106
130	127.525	2.47451
112	109.665	2.33441
95	96.685	-1.68502
85	87.2508	-2.25076

202 B

*** RESULT AFTER 10 ITERATIONS , ESQUARED = 24.5456 ***

INITIAL	CORRECTED	DIFFERENCE
285	282.923	2.07714
245	247.147	-2.14715
213	215.935	-2.93548
190	188.705	1.29437
167	164.949	2.05041
145	144.224	.775878
126	126.142	-.1427
110	110.368	-.368041
96	96.6057	-.605743

Figure 2(b)

Facsimile of curve fitting print-out.

NAME	K	CEQ	CO	CF	ERROR
SUB 202 A	-3•19107	62•1529	384•502	84	95•7991
202 B	-1•36476	2•49078	323•93	83	24•5456
203 A	-2•26953	76•7344	360•218	80	66•6595
203 B	-2•27587	52•5674	396•698	77	39•4108
204 A	-4•0634	39•2109	388•409	71	98•9039
204 B	-3•75166	34•692	493•051	72	12•6944
205 A	-2•98314	52•6671	294•456	72	98•159
205 B	-2•86463	49•5854	351•732	76	74•8244
206 A	-1•00816	-16•3673	257•582	75	12•7534
206 B	-1•66967	71•4713	334•356	91	125•977
207 A	-2•77772	50•7577	343•83	75	89•7447
207 B	-1•37892	-2•14378	297•608	73	86•8193
208 A	-3•69444	27•5788	392•151	80	8•01865
208 B	-3•54372	57•8894	393•35	89	129•469

Figure 3(a)

Cumulative print-out of derived functions.

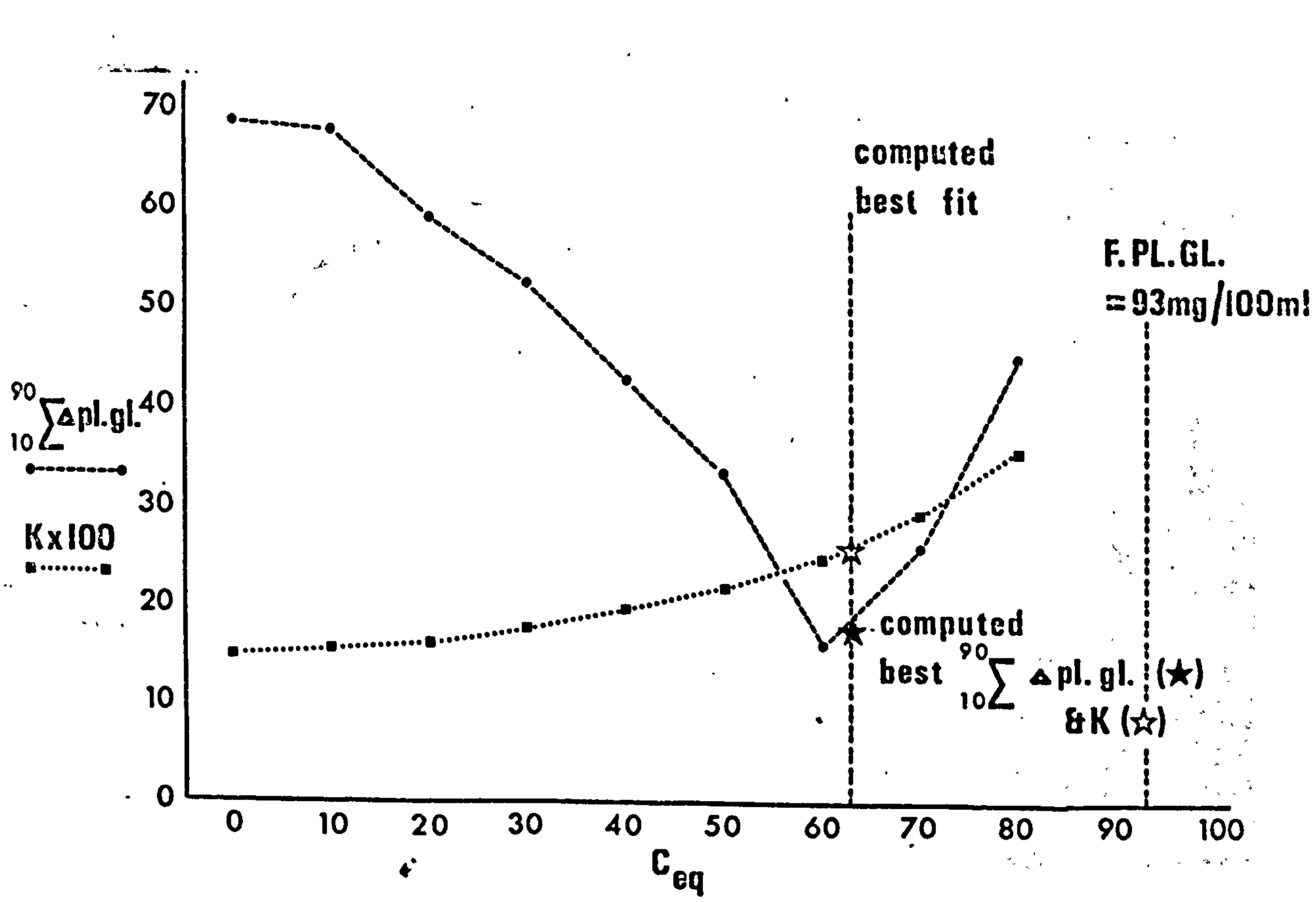


Figure 3(b)

Graph confirming validity of method.

I feel that the introduction of the parameter C_{EQ} destroys the simple concept of diabetes being characterised by low k values, in that some clearly abnormal intravenous tests are best described by high values of both k and C_{EQ}. This implies normal assimilation of glucose in the presence of increased mobilisation. This concept may be a useful basis for further studies on the abnormalities of carbohydrate tolerance in various types of diabetes.

Indeed it seems possible in ignoring the constant term C_{EQ} in the first order equation or equating it with the fasting glucose value one may be overlooking a physiologically significant parameter, which could possibly be related to glucose output from the liver independent of an uptake which is proportional to blood glucose concentration.

Franckson and his colleagues (1962) demonstrated that following intravenous glucose there was a reversal of the normal hepatic balance and glucose was taken up by the liver. The role of the other tissues in assimilating the injected glucose has received little attention. Franckson et al. pointed out that since the speed of assimilation appears to remain constant (in that the plot of log glucose against time gives a straight line), the serum insulin activity should not show marked variations during this exponential phase. They studied the insulin response to intravenous loading, using a rat epididymal fat pad method of measuring insulin-like activity; they found "a transient increase in insulin-like activity, of only slight importance."

Many workers had observed that plasma insulin rises after intravenous glucose and Samols (1965) showed that the rate of disappearance of glucose from the blood following intravenous injection appeared to correlate with the initial plasma insulin response and claimed that the insulin response to injected glucose was important in determining the rate of glucose disappearance.

There has been much debate over the relative advantages and disadvantages of oral and intravenous glucose tolerance tests (see Duffy, Phillips and Pellegrin 1973, for a good review). In favour of the oral test is the fact that it is easy to perform and most workers have an idea of what they consider to be a normal response. However variations in gastric emptying and intestinal absorption are unpredictable and uncontrollable. Gastrointestinal disease may either flatten the curve, as in some malabsorption states, or increase the height of the curve, as with the rapid gastric emptying following gastrectomy. Furthermore the rising and falling limbs of the oral curve do not reflect the true rates either of absorption of glucose or its disposal since the two processes are coincident over much of the curve (Duncan 1956).

The main advantage of the rapid intravenous glucose tolerance test is that an accurately measured load of glucose is administered in a known time. The result can be expressed fairly precisely in a form which allows a comparison of tests performed under different circumstances. Such a comparison is aided by the fact that the results of duplicate tests in the same individual under standard conditions show no significant difference on the two occasions (Duncan 1956). The main disadvantage of the intravenous test is that concentrated glucose has to be injected; this renders it less suitable for children and local leakage and mild plebitis may occasionally occur. When one compares the pros and cons of the two methods, oral and intravenous, it is easy to sympathise with Duncan (1956) who wrote "it is surprising indeed that the relatively crude oral test is so commonly employed when a much more accurate index of glucose tolerance is desirable."

However, the assumption that has been made, and still is, made in studies comparing the oral and intravenous methods of testing glucose tolerance is that they stress the same mechanisms of glucose assimilation and that there is no profound difference in the metabolism of glucose

administered by one or other route. Discrepancies between the tests have usually been attributed to the effects of gastric emptying or intestinal absorption. It has, however, been suggested (McIntyre et al. 1970) that the anatomical situation of the liver is important in the modification of the response to oral glucose.

This study is a detailed consideration of a mathematical model that may yield information about the differences which exist between the responses to oral and intravenous glucose and the mechanisms which may be responsible for these differences. However, before description of such a model further consideration will be given to the biochemical mechanisms that could be involved in the hepatic regulation of blood glucose concentrations.

CHAPTER 3

THE BIOCHEMICAL MECHANISMS INVOLVED IN THE CONTROL OF GLUCOSE UPTAKE AND RELEASE BY THE LIVER

As discussed in the last chapter the capacity of the liver to maintain the blood glucose concentration at an optimal level when the dietary carbohydrate intake is low was first recognised by Claude Bernard.

However, the first detailed biochemical experiments on blood glucose regulation by the liver were performed by Soskin et al. (1938) who demonstrated that the dog liver, in situ, would take up glucose when perfused with hyperglycaemic blood, and would release glucose when the glucose concentration of the perfusate was low.

Brady, James and Farrar (1949) confirmed these observations, using catheterisation techniques, as did Searle and Chaikoff (1952), who used an isotopic technique to estimate glucose production, and showed that hyperglycaemia inhibited hepatic glucose release. Cahill et al. (1959) proposed that the balance between hepatic glucose uptake and hepatic glucose release was primarily determined by the relative activities of hexokinase (EC 2.7.1.12) and glucose-6-phosphatase (EC 3.1.3.9). Of the two enzymatic activities hexokinase would probably be more responsive to changes in glucose concentration in the blood.

Subsequent studies by Herrera et al. (1966) have suggested that in the isolated perfused liver the rate of incorporation of carbon atoms from alanine into glucose can be significantly altered by the glucose concentration of the perfusion medium. Exton and Park (1967) on the other hand did not observe any marked effect of perfusate glucose concentrations on the incorporation of carbon from lactic acid into

glucose in the isolated perfused rat liver.

To explain the physiological functioning of the liver in biochemical terms it is necessary to identify the reactions that can be described as regulatory as far as hepatic glucose release and uptake are concerned.

It is improbable that transport across the hepatic cell membrane is regulatory for transfer of glucose across the membrane is an equilibrium process. This is demonstrated by the fact that the membrane is highly permeable, the intracellular concentration is similar to the extracellular concentration, and that extracellular ^{14}C-glucose rapidly equilibrates with intracellular glucose independent of the direction glucose flux.

The obvious candidates for regulatory control are, as pointed out by Cahill et al. (1959), hexokinase, the enzyme that phosphorylates glucose, and glucose-6-phosphatase, the enzyme that hydrolyses glucose-6-phosphate to yield glucose. Both enzymes catalyse non-equilibrium reactions; the mass action ratios for these reactions are smaller than the equilibrium constants and the maximal catalytic activities of these enzymes are low compared with the other enzymes of the glycolytic pathway (see Table I).

Glucose uptake by the liver

The phosphorylation of glucose may be catalysed by one of several enzyme systems (Cahill, et al. 1959), classified by Katzen (1967) according to their Km for glucose, as type (1) Km 10^{-5}M; type (2) Km 10^{-4}; type (3) Km 10^{-6} and type (4) Km 10^{-2} hexokinase. The enzymes with the low Km types, 1 to 3, are fully saturated at very low plasma glucose concentrations. Type 4 exhibits $\frac{1}{2}$V max with a plasma glucose concentration of 10 mmol, which would permit an increased hepatic glucose uptake with an increasing hepatic portal blood glucose concentra-

Enzyme	Enzyme activity in µmol/min/g fresh weight at 37°C
Hexokinase	0.5
Glucokinase	2.0
Glucose-6-phosphatase	12
Glucosisomerase	280
6-phosphofructokinase	5.0
Hexose-diphosphatase	20
Fructose biphosphate aldolase	6.2
Glyceraldehyde phosphate dehydrogenase	150
3-phosphoglycerate kinase	130
Enolase	53
Pyruvate kinase	37
Lactate dehydrogenase	230
Pyruvate carboxylase	8.3
Phosphoenolpyruvate carboxykinase	13

Table I Maximal activities of the glycolytic and gluconeogenetic enzymes in the hepatocyte. Data from Scrutton and Utter (1968).

tion over a wide physiological range. It is the differences between the properties of this latter enzyme, glucokinase (EC 2.7.1.12), and the other hexokinases that provide the basis of a theory of regulation uptake which is consistent with the physiological function of the liver. After a meal containing carbohydrate the concentration in the portal blood increases from about 5mmol/l to 12mmol/l or more and because glucose transport is an equilibrium process the intracellular glucose rises in parallel. This increase in concentration of glucose produces a marked rise in glucokinase activity and hence of hepatic glucose uptake. The high Km of glucokinase for glucose is necessary for operation of this control step, for hexokinase would be saturated at 1mmol/l glucose and any change above that concentration would have no effect upon its activity. Also the absence of product inhibition of glucokinase ensures that hepatic accumulation of glucose-6-phosphate does not interfere with glucose uptake.

Stimulation of glucokinase activity thus leads to an increased concentration of hepatic hexose phosphates, which in turn leads to an increase in activity of 6-phosphofructokinase (EC 2.7.1.11) and UDPG glucosyltransferase (EC 2.4.1.11). Thus increase in glucokinase activity will provide a control system in which increase in hepatic portal glucose concentration increases hepatic uptake and stimulates glycolysis and glycogen synthesis.

Thus, hepatic glucokinase can be seen to be a mechanism that by-passes the regulation of glycolysis as seen in muscle, in which product inhibition of hexokinase has a dominant role. The importance of this mechanism is great in that it permits the liver to regulate glucose uptake depending upon the hepatic concentration of glucose, and also facilitates the conversion of glucose residues to glycogen or/and triglyceride (see Figure 4).

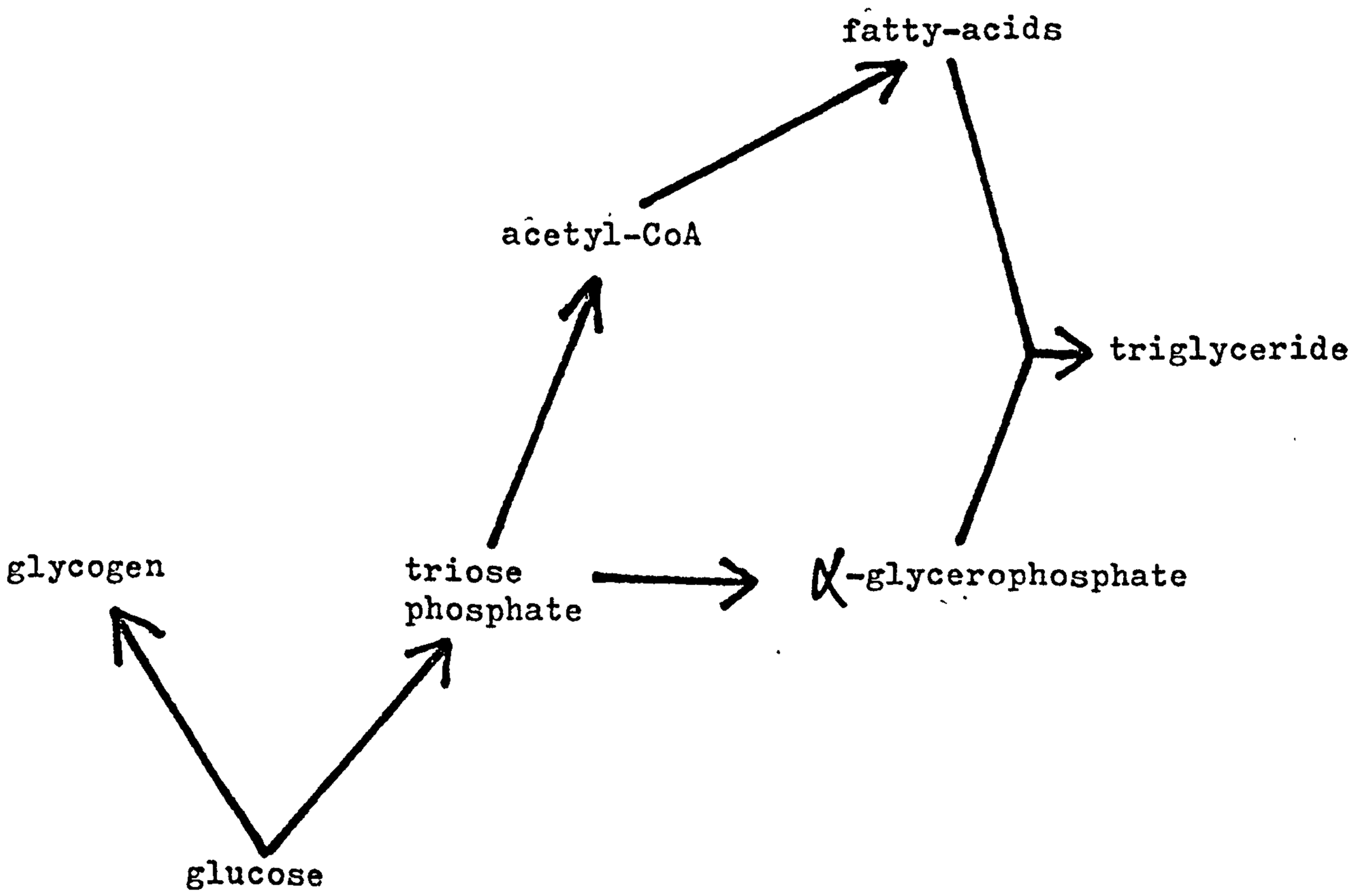


Figure 4

The alternative pathways for conversion of glucose residues to glycogen or triglyceride in the liver.

Evidence for the involvement of glucokinase in this fashion is as follows:

i) glucokinase is found in liver tissue only, and furthermore liver is the only tissue subjected to large variations in extracellular glucose concentration because of its proximity to the absorptive area of the gut;

ii) the activity of glucokinase varies markedly with the dietary state, and these changes appear to be due to changes in concentration of enzyme-protein probably controlled by insulin (Walker 1966).

There is no doubt that the enzyme activity increases under conditions when the plasma insulin is high, as after feeding, and decreases when the plasma insulin level is low as in starvation or in diabetes mellitus.

Sharma, Manjeshwar and Weinhouse (1964) and Katzen (1967) have demonstrated reduced activity in starvation, and Borrobaek et al. (1970) the similar effect of low-carbohydrate diet. Several hours after re-feeding with carbohydrate following carbohydrate deprivation, glucokinase activity increases to normal levels (Borrobaek et al. 1970; Salas, Vinuela and Sols 1963). Furthermore, Hornichter and Brown (1969) demonstrated that following starvation there is a direct relationship between hepatic glucokinase activity and glucose tolerance.

McCraw (1968) has also shown that in the isolated perfused rat liver glucose utilisation is reduced following starvation, whilst Landau, Leonards and Barry (1961) have demonstrated that in the dog sensitivity of the liver to changes in blood glucose concentration is considerably decreased by prior carbohydrate restriction. Furthermore, during glucose infusion in animals on a high carbohydrate diet blood glucose levels increased very little only reaching 100-130 mg/100 ml, yet net hepatic glucose storage (as glycogen) occurred; in contrast,

in carbohydrate-deprived animals there was a much greater rise in glucose concentrations, and it was only at levels of 150-200 mg/100 ml that hepatic glucose uptake was observed.

iii) Glucokinase is not present in the young rat before weaning. Thus while fed by the mother hepatic uptake is controlled; post-weaning when the dietary intake of carbohydrate will vary glucokinase becomes necessary for control of hepatic uptake.

There is an alternative glucose phosphorylating system present in liver tissue, catalysed by glucose phosphotransferase (EC number not given) but this enzyme is only effective at very high glucose concentrations, 30mmol/l and upwards (McCraw 1968).

Regulation of glucose uptake and release by the liver

The properties of glucokinase described above can explain the mechanisms for glucose uptake by the liver but there is a problem when release of glucose is considered. Liver releases glucose when the concentration of glucose in the portal vein falls below an optimal concentration of about 4.5mmol/l. Yet, at this blood glucose concentration the activity of glucokinase *in vitro* is relatively high (about 25% of maximal activity). There are no known properties of the enzyme suggesting inhibition when the portal blood glucose concentration falls below normal. Even the isolated perfused rat liver is able to respond to changes in glucose concentration (Williamson et al. 1965) and in these circumstances it is only the perfusate glucose concentration that is the controlling factor for uptake or release of glucose.

A regulation mechanism must therefore be postulated that involves glucokinase and glucose-6-phosphatase and their ability to respond to changes in glucose concentration. A small fall in glucose concentration can produce a complete change in the rate and direction of glucose flux and metabolism by the liver, so it is improbable that glucose alone can

act as controller by regulating the enzymes.

A metabolic intermediate could act in this way but to provide adequate activation or inhibition an amplification system would be necessary. There is no evidence for such a regulator or amplification system. In the absence of direct corroborative evidence two hypotheses have been postulated to account for regulation of glucose uptake and release by the liver, namely substrate cycling and compartmentation.

Substrate cycling

Simultaneous activity of glucokinase and glucose-6-phosphatase will result in glucose conversion to glucosa-6-phosphate (with ATP hydrolysis) and glucose-6-phosphate hydrolysis back to glucose (Figure 5). It is possible for such a substrate cycle to be very sensitive to changes in concentration of a regulator molecule, and could explain the physiological role of the liver in releasing and taking up glucose. It is necessary for efficient working that glucokinase activity *in vivo* would exceed that of glucose-6-phosphatase and that the level of hepaticellular glucose-6-phosphate be relatively constant. Such a theory proposing that glucokinase activity varies according to the glucose concentration and thus regulates *direction* and *flux* of glucose metabolism was put forward by Newsholme and Gevers (1967). They suggested that as the glucose concentration approached that for saturation of glucokinase (about 50mmol) the net hepatic uptake of glucose is given by V_{max} for glucokinase minus a constant rate which represents the activity of glucose-6-phosphatase. As glucose concentration falls and approaches normal, the activity of glucokinase with fall concommitantly until it approaches that of glucose-6-phosphatase. Around 4.5mmol glucose the activities of both enzymes are equal and there is no net release or uptake of glucose by the liver. As the concentration of glucose falls below this level the activity of glucokinase falls below that of glucose-6-phosphatase and hydrolysis of glucose-6-phosphate will

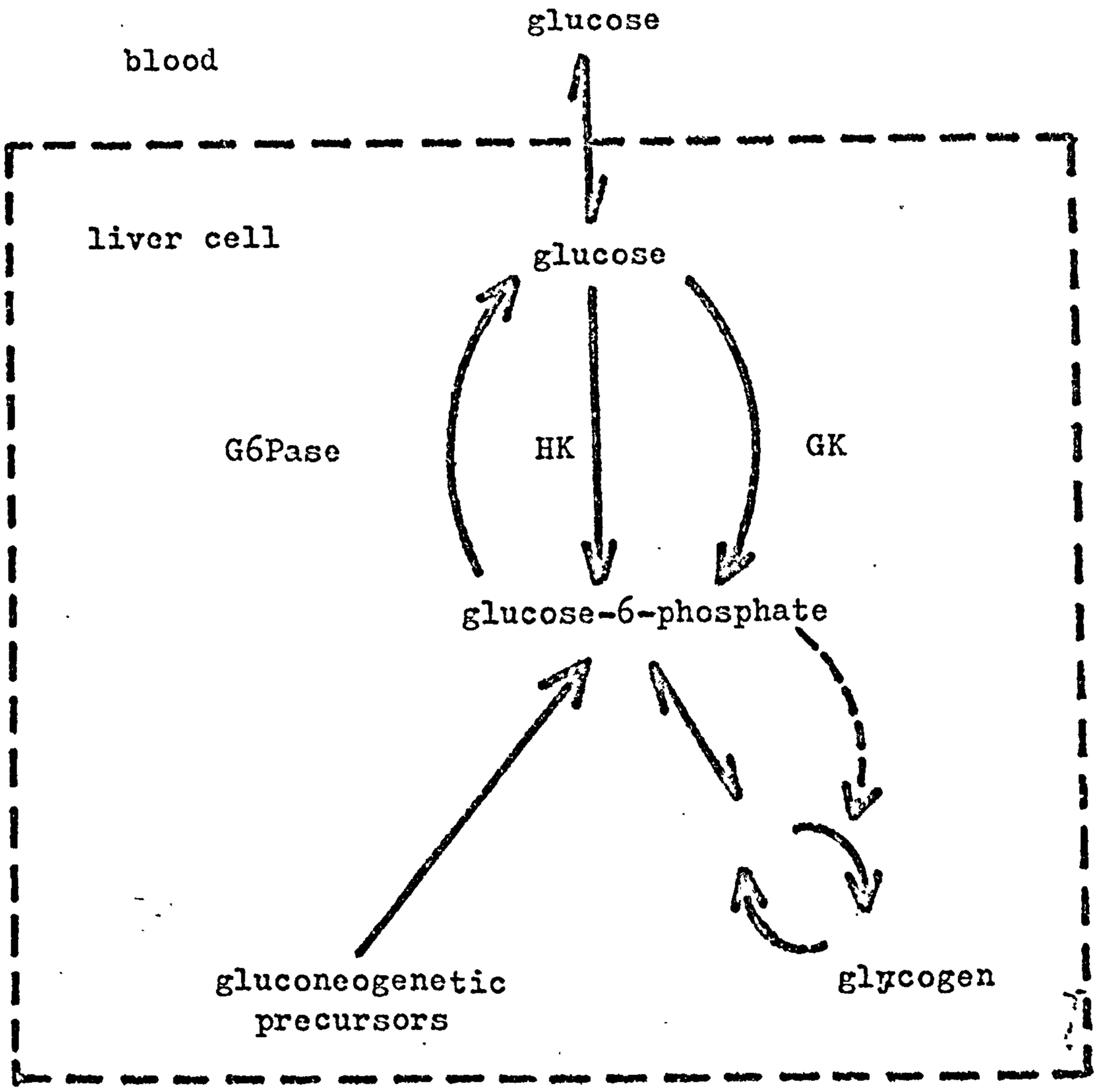


Figure 5

Enzymes involved in glucose, glucose-6-phosphate interrelationships in the liver (see page 41).

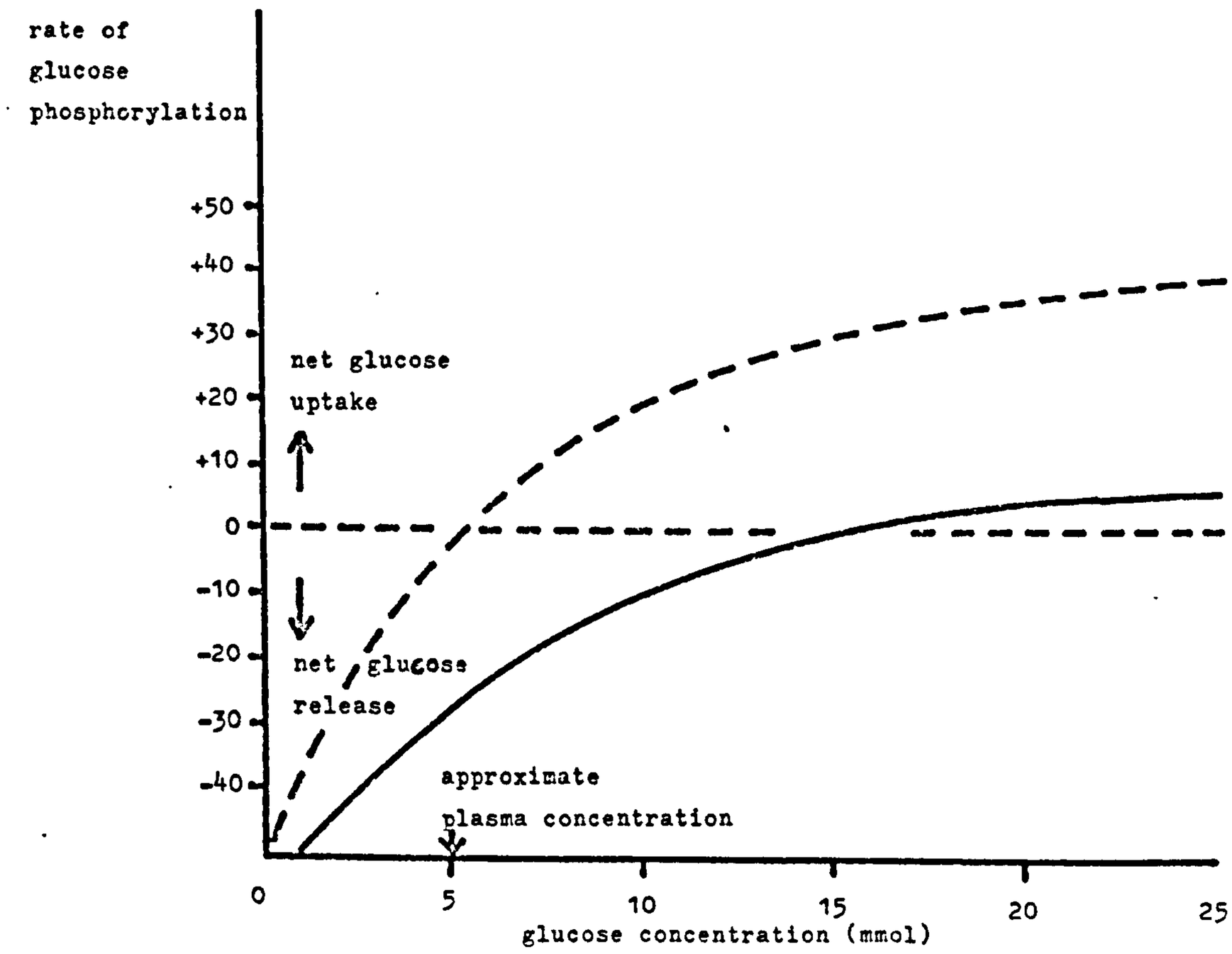
GK = glucokinase; HK = hexokinase; G6Pase = glucose-6-phosphatase

occur, with release of glucose into the extracellular space.

Thus, changes in glucose concentration will control the direction of glucose flux and hence metabolism. This hypothesis provides a biochemical explanation for the observations that the rate of uptake or release of glucose is proportional to the degree of hyper - or hypo-glycaemia (see Figure 6). Such a mechanism is simple, requires no specific regulator molecules and relies on glucose concentration only, but there is no concrete evidence to support or refute its existance. It does depend on a near constant glucose-6-phosphatase activity and hence constant glucose-6-phosphate concentration. Thus the flux into the hexose monophosphate pool must be increased when the glucose release rate of the liver increases in order to maintain a constant level of glucose-6-phosphate. Such a situation could be achieved by increased formation of glucose-6-phosphate by gluconeogenesis, or alternatively by control of glycogenolysis in parallel with glucose-6-phosphate hydrolysis (a sort of 'control in series'). Either of these mechanisms would implicate modulation of these processes by further hormonal control.

Many studies have been directed towards determining whether there is hormonal control of hepatic gluconeogenesis and much argument devoted, especially to the protective role of insulin and if so by what mechanisms and at what steps. The many volumes of Advances in Enzyme Regulation (ed. G.Weber; 1963 to date) bear witness to this. Numerous enzyme reactions have been implicated as it has been demonstrated, principally in vitro, that they may be altered in activity as a result of hormonal administration or deficiencies. The technical expertise involved in such work cannot be denied, but the relevancy of the observations to physiology must often be doubted.

However, there is evidence, that the control of hepatic gluconeogenesis can be accomplished by varying the rate at which substrate is presented to



A theoretical plot of glucokinase activity (represented by the rate of phosphorylation of glucose) against plasma glucose concentration.
It can be seen how the alteration of glucokinase activity allows the liver to vary the rate of uptake or release of glucose from or to the plasma.

Figure 6

the liver (Exton and Park 1967; Herrera et al. 1966); that is the rate of gluconeogenesis is proportional to the concentration of gluconeogenic precursors, and furthermore can be altered very rapidly, within seconds of change of substrate concentration. There is overwhelming evidence that the provision of gluconeogenic substrate from peripheral tissues is a function of hormonal activity within those tissues.

Thus, enzyme activity may be changed by hormonal alterations which in turn will alter the gross limits of maximum and minimum rates for saturating concentrations of substrate. Evidence for this view is that normally induced alterations invariably occur over a time-scale of hours; whereas the alterations in rates of gluconeogenesis occurs within seconds of change of substrate concentration.

An interesting further confirmatory phenomenon is that free fatty acids by providing for the liver's energy requirements (Herrera et al. 1966; Williamson et al. 1966) spare the gluconeogenic precursors from oxidative processes and make them available for glucose formation. Thus at any given glucose precursor concentration an increase in the free fatty acid concentration will increase the rate of gluconeogenesis. These observations suggest that whilst the minute-to-minute regulation of the glucose steady-state is a function of the liver, the liver in its turn is particularly dependent on hormones, especially insulin, working on peripheral tissues and thereby regulating the rates of precursor release, and availability of oxidative substrate. The control in series, that is activation of the glycogen phosphorylase mechanism in parallel with glucose-6-phosphatase, may be a very important mode of provision of glucose-6-phosphate.

Compartmentation theory

This theory proposes that glucokinase and glucose-6-phosphatase are not simultaneously active because they are compartmented and the products

and substrates for the two enzymes are not able to mix (Figure 7). That is glucose-6-phosphate may function as a precursor for glycogen synthesis or for glycolysis, but not as a substrate for glucose-6-phosphatase. The substrate for the latter enzyme is produced by glycogenolysis or gluconeogenesis, and its hydrolysis yields glucose that is released into the blood and does not have contact with glucokinase.

This theory is not supported by direct evidence though indirect evidence for it is provided by London (1966), Heath and Threlfall (1968) and Gumaa, McLean and Greenbaum (1971). The real problem associated with this theory is to identify the intracellular location of enzymes. Glucokinase is cytoplasmic whereas glucose-6-phosphatase is found on the endoplasmic reticulum, but this is not evidence necessarily of a functional separation. To be satisfactory as a theory complete physical separation of the glycolytic and gluconeogenic pathways must be postulated, otherwise the problems of substrate recognition become insuperable. All in all the substrate cycling appears to be the most attractive theory. It is anticipated that this mode of control will be one of those factors that modelling will highlight.

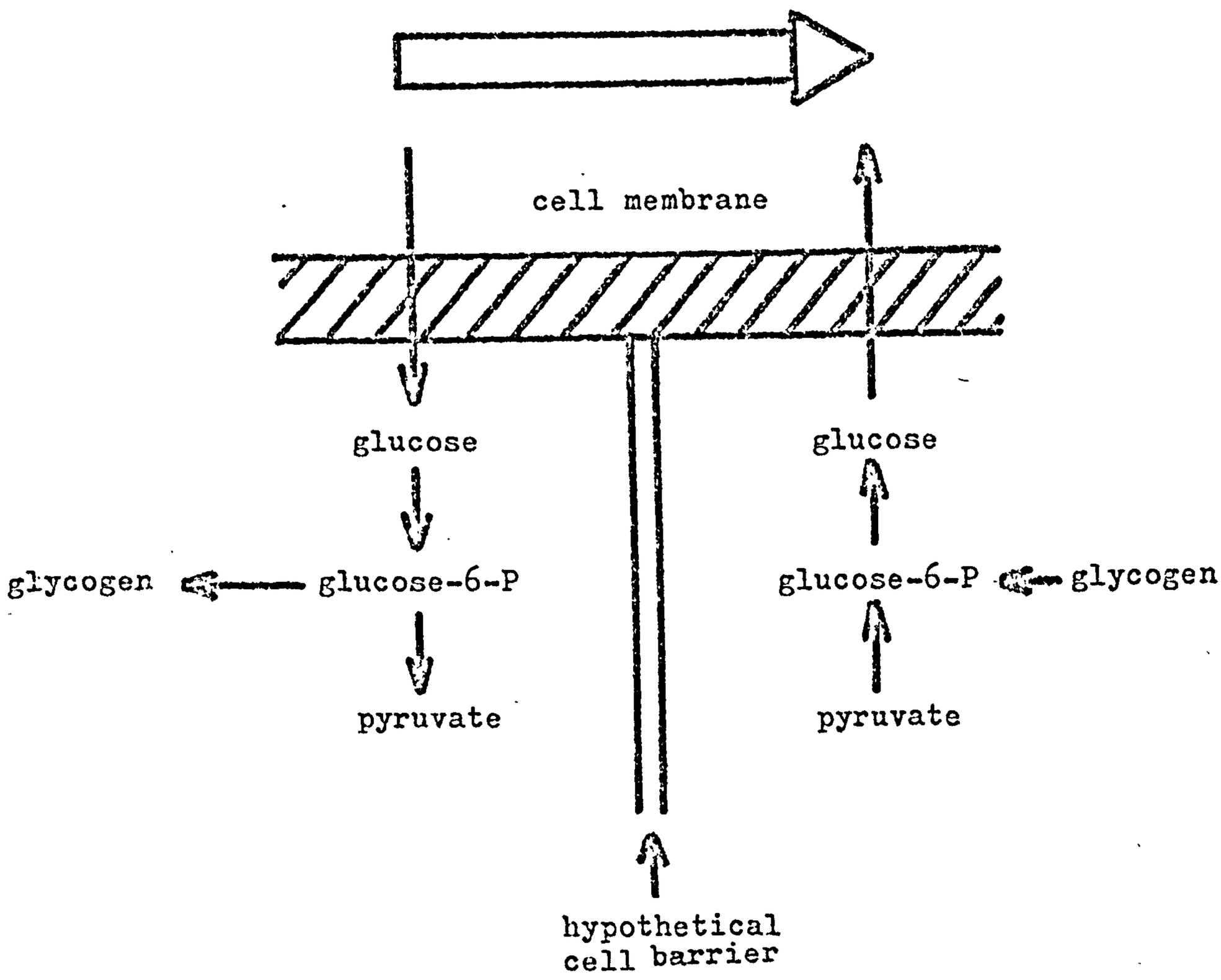


Figure 7

Diagrammatic representation of 'compartmentation' of glycolytic and gluconeogenetic pathways.

CHAPTER 4
A CRITICAL REVIEW OF SOME MODELS OF BLOOD GLUCOSE REGULATION

Numerous authors have attempted to explain the relationships between the concentration of glucose and insulin in plasma, both of animals and man, especially after the steady-state had been disturbed by oral or intravenous glucose. However, as has been pointed out by Atkins (1971) nearly all these models were inadequate. They could be faulted for one or more of the following reasons:

i) the proposed model was too complex to be fitted to the amount of data available;

ii) the results available were for plasma glucose only;

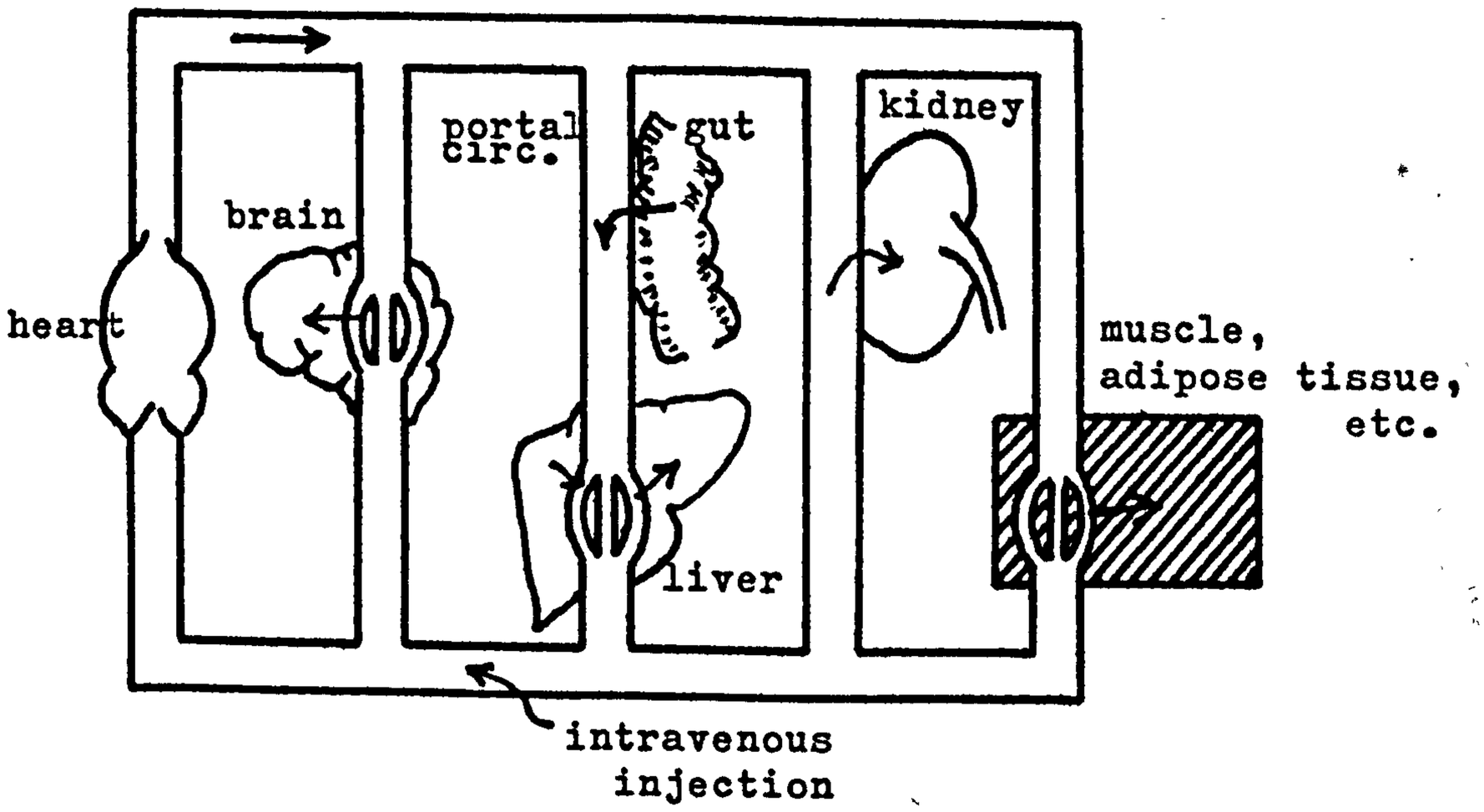
iii) the time-scale of the experiments providing data was too short;

iv) the models were not consistent with known physiological and biochemical data.

Atkins (1971) in the light of these criticisms went on to compare 24 possible theoretical models, all relatively simple. He found 6 different models conformed fairly well to three sets of data obtained from the literature. In other words none was unique.

It is simplification of a very complex process (Figure 8) that yields the problem of non-uniqueness. Simplification requires the lumping of several distinct organ level processes as a single quasi-linear process and this makes physiological interpretation of simulation results very difficult. The introduction of versatile digital computing methods has, however, made possible modelling that is increasingly isomorphic with <u>in vivo</u> systems, and which therefore include the non-linearities which normally control the dynamics of the glucose regulatory system. The conceptual development of this approach is discussed in this chapter.

peripheral circulation



peripheral circulation

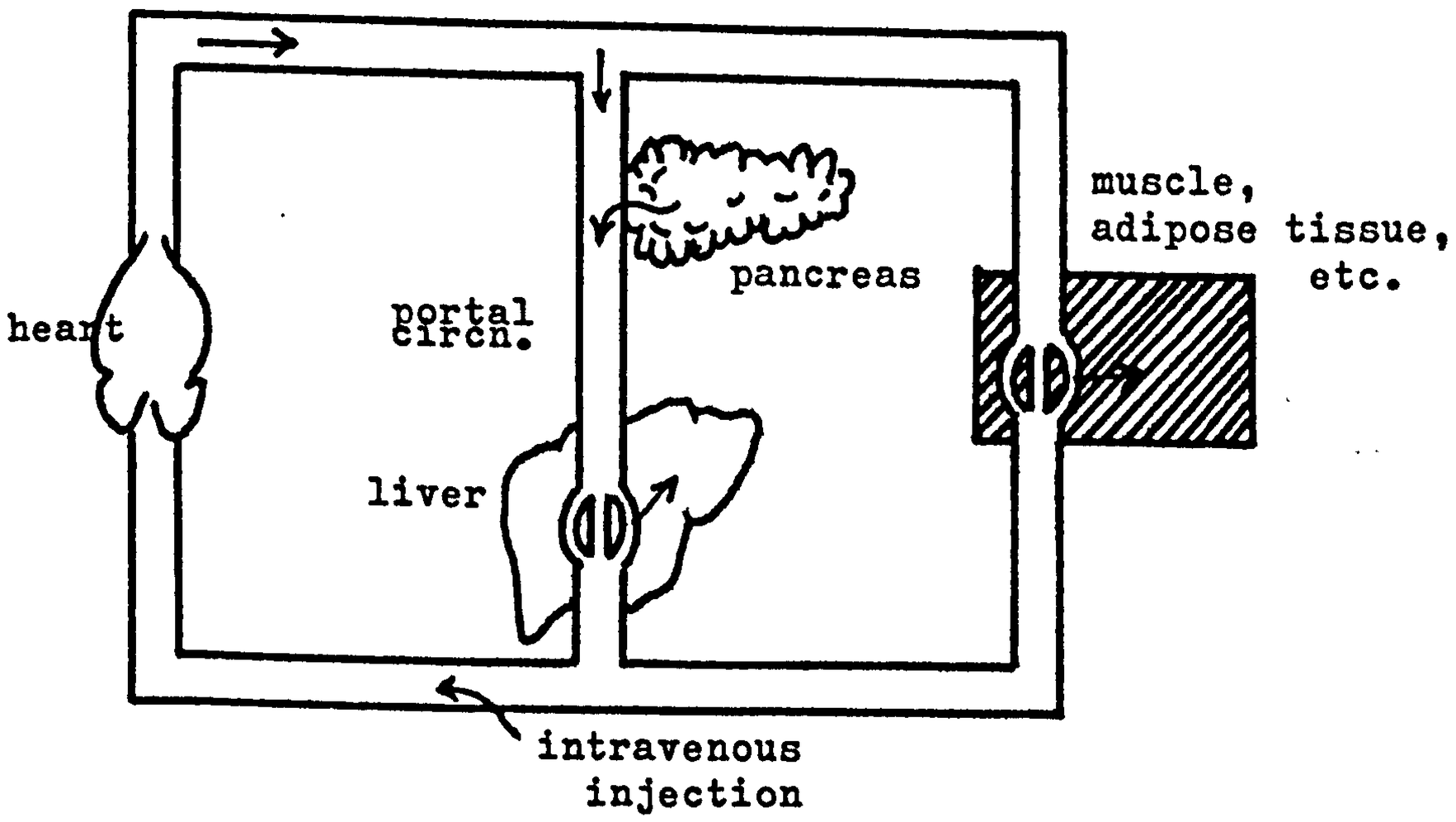


Figure 8

Pictorial representation of the circulatory routes of glucose (top) and of insulin (bottom).

Probably the first attempt to illustrate how the concepts of control systems engineering may be applied to the study of glucose homeostasis was that of Goldman (1960). In a qualitative fashion he summarised the major metabolic sub-systems and controlling hormones relevant to glucose regulation and represented their inter-connection in a block diagram suggestive of a multi-loop feedback control system (see Figure 9). Goldman did not attempt to develop his model in any more detail, but discussed the eventual application of modelling efforts in characterizing clinical abnormalities and in endocrine system research. Assuming that glucose regulation can be viewed as a classical regulating system, Goldman emphasised the need for new experiments to understand the physiological mechanism of set-point establishment and to identify which possible modes of control exist in this physiological system. Although Goldman's work does not enhance our physiological knowledge of glucose metabolism per se, it was the first detailed mathematical modelling of the system by identifying the major sub-systems involved and their logical interconnection in a systematic manner consistent with standard control system terminology.

The next published work is that of Bolie (1961) illustrating the use of analog computers in simulation of mathematical representations of glucose regulation. The first model treats the interaction of liver, kidney, pancreas, insulin and glucose in two compartments, the vascular and the extravascular.

The model is a gross simplification of the glucose regulating system, treating only one controlling hormone and being strictly linear except for a renal threshold. It qualitatively reproduces insulin and glucose behaviour after simulated 6-minute infusions of glucose and insulin of 50g and 2 units respectively. The model is represented by equations (4.1) to (4.4) :

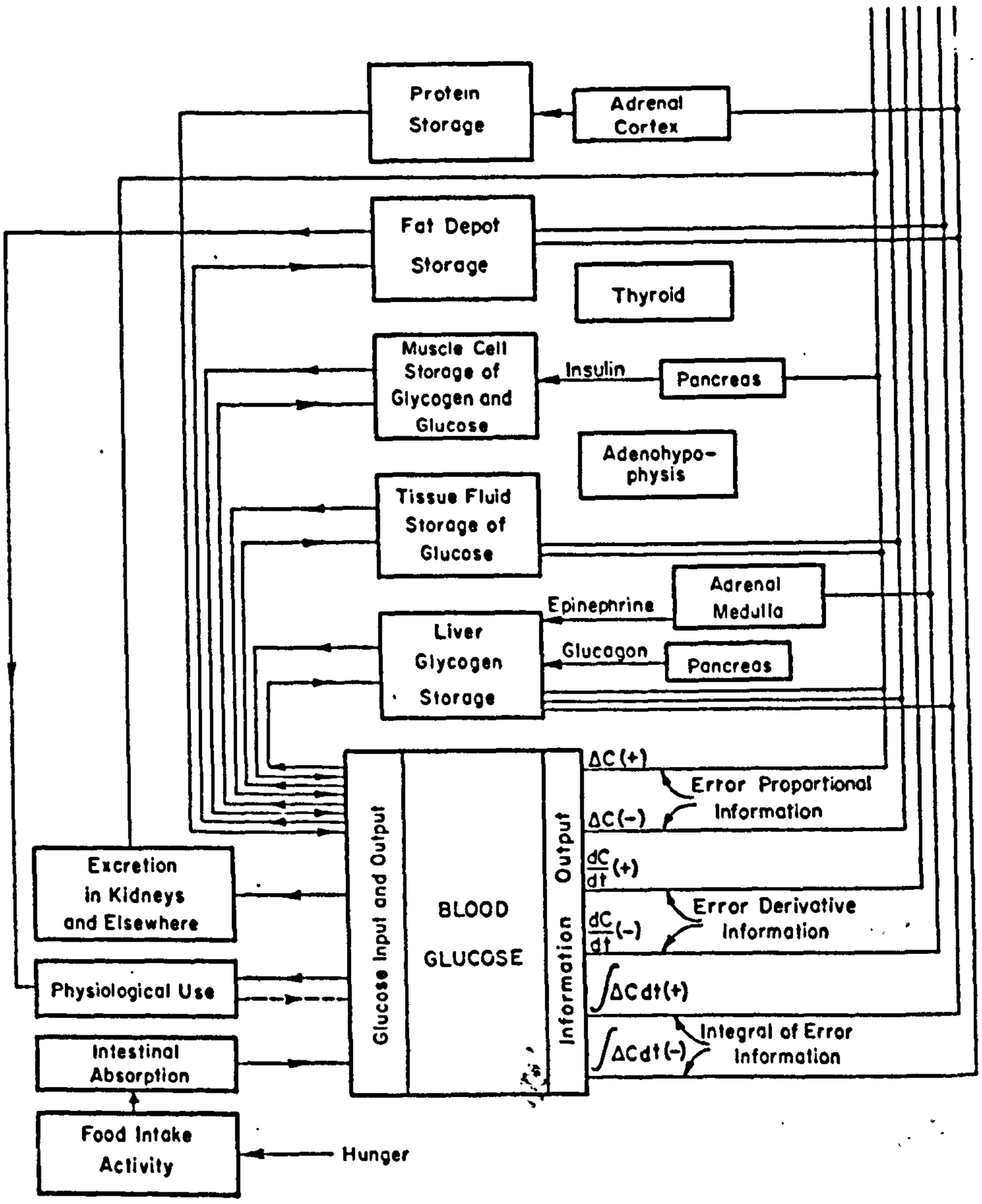


Figure 9 : Certain aspects of the blood glucose control system as described by Goldman (1960).

$$V_B \dot{I}_v = I_i + (I_e - I_v) P_I + K_1 G_v - K_2 I_v \qquad (1)$$

i.e. the rate of accumulation of insulin in the blood stream equals the rate of injection plus the rate of transfer from the extravascular compartment plus the rate at which it is released from the pancreas minus the rate at which it is degraded in the vascular compartment.

$$V_I \dot{I}_e = (I_v - I_e) P_I - K_3 I_e \qquad (2)$$

i.e. the rate of accumulation of insulin in the extravascular insulin space equals the rate of transfer from the vascular space minus the rate at which it is degraded in the extravascular space.

$$V_B \dot{G}_i = G_i + (G_e - G_v) P_G - f(G_v) - K_4 I_v - K_5 G_v \qquad (3)$$

i.e. the rate of accumulation of blood glucose equals the rate of injection plus the rate of transfer from the extravascular compartment minus renal excretion and storage in liver.

$$V_G \dot{G}_e = (G_v - G_e) P_G - K_6 I_e - K_7 G_e \qquad (4)$$

i.e. the rate of accumulation of extravascular glucose equals the rate of transfer from the vascular space minus tissue utilisation.

Known non-linear effects have been linearised and represented by the constants K_1 to K_7. The renal nonlinearity $f(G_v)$ consists of a threshold and constant gain. Although the model does not yield any new information about glucose regulation, it does illustrate on a limited scale the use of simulation in verifying the consistency of a proposed model.

Bolie's second paper (1961) neglects renal excretion and lumps the vascular system and extravascular system into one compartment. The model was then reduces to two first-order linear differential equations:

$$\dot{I} = \frac{I_i}{V} - \alpha I + \beta G \qquad (5)$$

$$G = \frac{G_i}{V} - \gamma I - \delta G \qquad (6)$$

where α represents the sensitivity of insulinase (EC 3.4.99.10) activity to insulin concentration, β represents the sensitivity of pancreatic insulin output to glucose concentration, γ represents the combined sensitivity of liver glycogen storage and tissue glucose utilisation to elevated insulin concentration, and δ represents the combined sensitivity of liver glycogen storage and tissue glucose utilisation to elevated glucose concentration.

The parameters of this model were derived from data obtained from various sources, in some cases values were "averaged" across species ! No attempt was made to verify the behaviour of the model by predictability of experiments. It is evident that at best the model parameters are chosen optimally with respect to one experiment.

The next published model of glucose regulation was that of Seed, Acton and Stunkard (1962). The model related liver, kidney, brain, pancreas, vascular and extravascular compartments, and a substance Z , presumably related to insulin, in terms of three dependent variables connected by piecewise linear ordinary differential equations. The equations represent respectively, the rate of change of glucose in a "fast" compartment (mainly plasma), the rate of change of glucose in a "slow" compartment (intracellular), and the rate of change of substance Z in the liver. The expressions in square brackets are zero unless the condition note below each bracket is satisfied.

$$G_f = -D_f G_f + D_s G_s - L_z G_f - M_a G_f - B - (M_f G_f - m_f) - (R G_f - r) + (g_p - P_g Z) + (I)$$

$$(G_f > L_f) \quad (G_f > L_v) \quad (Z < L_n) \quad (t < I_T)$$

$$\dot{G}_s = D_f G_f - D_s G_s - (M_s G_s - m_s) \qquad (8)$$

$$(G_s > L_s)$$

$$\dot{Z} = -c_z - C_z Z + F_z G_f \qquad (9)$$

These equations were simulated by trial and error parameter adjustment showed qualitative agreement over a limited interval with some kind of an "average" of plasma glucose after a 25g infusion taken over 70 normal subjects (see Figure 10). These data were obtained by Amatuzio et al. (1953). However, this method of model verification was found to yield physiologically unrealistic parameters.

These investigators then expanded the model to include two new compartments, red blood cells and plasma. The equations represent, respectively, the rate of change of plasma glucose, fast compartment glucose, slow compartment glucose, red blood cell glucose, and substance Z in liver.

$$\dot{G}_p = (D_{pf} G_p - D_{fp} G_f) - (D_{ps} G_p - D_{sp} G_s) - KV_{G_c} \frac{G_p}{G_p + G_{\phi_p}} - \frac{G_c}{G_c + G_{\phi_c}}$$

$$- (P_g Z - p_g) - L_z G_f - (RG_p - r) - B + (I) \qquad (10)$$

$$(Z < L_p) \qquad (G_p > L_r)\ (t > L_T)$$

$$\dot{G}_f = (D_{pf} G_p - D_{fp} G_f) - M_a G_f - (M_f G_f - m_f)$$

$$(G_f > L_f) \qquad (11)$$

$$\dot{G}_s = (D_{ps} G_p - D_{sp} G_s) - M_b G_s - (M_s G_s - m_s)$$

$$(G_s > L_s) \qquad (12)$$

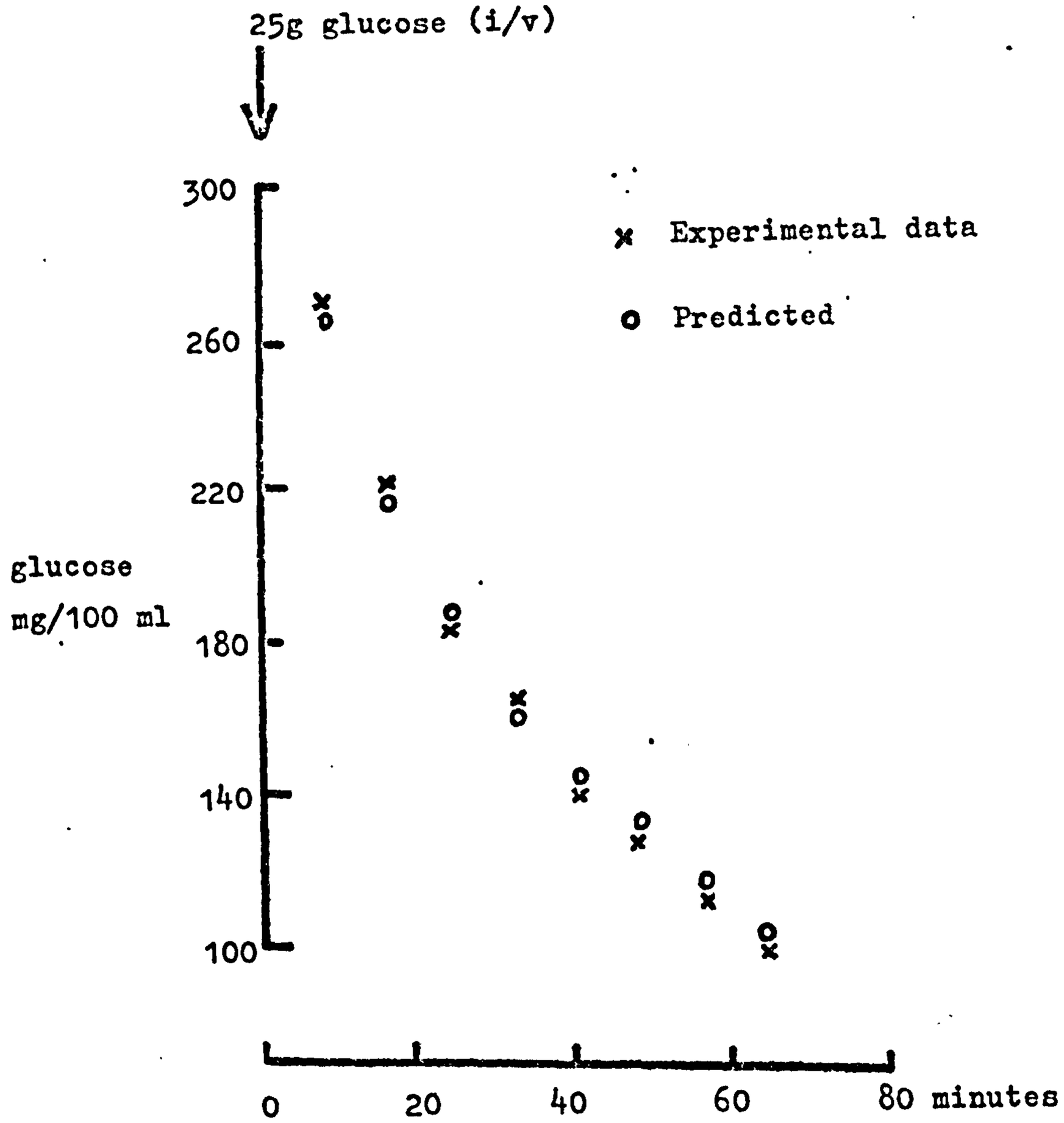


Figure 10 An intravenous glucose tolerance test curve of Amatuzio et al. (1953) compared with the predicted values from the model of Seed, Acton and Stunkard (1962).

$$\dot{G}_c = KV_{G_c} \frac{G_p}{G_p + G_{\not p_p}} - \frac{G_c}{G_c + G_{\not p_c}} \qquad (13)$$

$$\dot{Z} = F_z G_f - (C_z Z + c_z)$$
$$(Z > 0)$$

The authors attempted to substantiate all parameter values from published experimental results before computer simulations were undertaken. Again, roughly qualitative reproduction of experimental results was obtained, and it was concluded that more knowledge of glucose metabolism was required before a more refined model could be postulated. As hormone effects are not included in this work, the deficiencies of the model are substantial. As in the previous two efforts, the model is really a sophisticated attempt at curve-fitting.

In 1964 McLean published a brief summary of glucose homeostasis focussing primarily on Goldman's (1960) work. He attempted to incorporate nervous system effects explicitly in a modified system block diagram. However, owing to our very rudimentary understanding of mathematical representations of neural processes, successful modelling of such processes await the results of more basic research.

Bearing in mind the complexity of the glucose metabolic control system I have devised and shown in Figure 11 it can be seen that the above mentioned models can only be regarded as tentative attempts at achieving some kind of a mathematical representation for certain portions of the complete system. Common to all these efforts is a restricted emphasis on glucose metabolism, lipid and protein metabolism being neglected.

Although not representative of metabolic system modelling, some related work has been done on glucose tolerance test analysis by Ackerman and co-workers (see Ackerman et al. 1969 for a review of this approach). These workers sought to characterise a subject's response

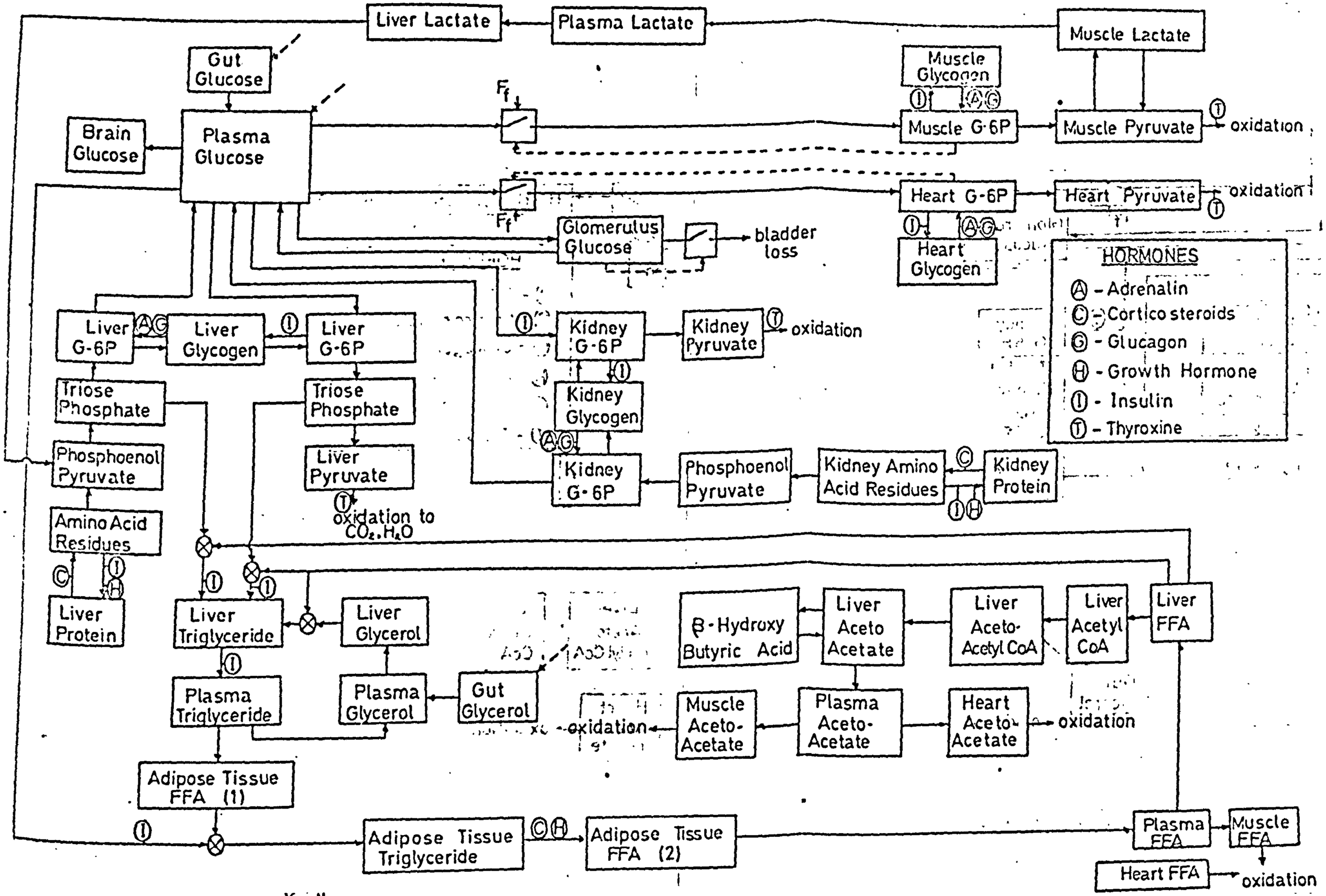


FIG 11. FUNCTIONAL MODEL OF GLUCOSE AND RELATED METABOLIC PATHWAYS

Figure 11

Functional Model of glucose and related metabolic pathways

to a glucose input by one parameter in an attempt to identify normal or abnormal system behaviour on the basis of the parameter value. Basically, their approach uses a linear second order model and the natural frequency of oscillation of the model is the parameter under study. The system variables are glucose and insulin concentration in blood. For the categorisation of a specific subject, an oral glucose tolerance test is performed, the blood glucose concentration being determined at timed intervals over a period of 180 min. The parameters of the model are fitted to this data in a least-squares sense. The resultant parameters then dictate a value for ω_0, the natural frequency of the glucose regulation model (presumably also that of the subject). On the basis of experiments on many subjects they concluded that classification of normals vs. diabetics in terms of ω_0 was comparable in reliability to previously used techniques, though they also recognise that borderline cases are difficult to classify.

Considering the complexity of the system however, it would be rather surprising if a useful tool could result from the least-squares fit of a second order equation to the coarsely sampled response of the metabolic system to an oral glucose load.

Early in 1967 Cerasi published the results of some work done in Sweden on analogue simulation of glucose regulation. The model represents insulin and glucose in one compartment and includes renal excretion, peripheral glucose uptake, and insulin secretion. The release of insulin in response to glucose concentration is represented in two phases, the release of stored insulin and the release of newly formed insulin. The model is not strictly linear, since the uptake rate of glucose by peripheral tissue, for example, is represented as proportional to the product of insulin and glucose concentrations, and a renal excretion threshold is included.

The author claims successful simulation of many oral glucose tolerance tests performed on humans in their laboratories. The intention of the author was to represent in a mathematical fashion the alterations in glucose and insulin concentration after a glucose load by means of a lumped model. However, it is apparent that the model is an insufficient representation of the glucose regulatory system, considering what is known today about the metabolic processes involved and their humoral controls.

A somewhat different approach has been taken by Kadish and his group. Owing to the complexity of the system depicted in Figure 11 and the paucity of experimental data that can suggest reasonably valid analytical models for the relevant sub-systems, these workers tried to construct models using as small a set of basic analytical blocks as possible with parameter values chosen for any specific process within a given class to accommodate the behaviour of that process. The real objective of this work is to demonstrate by means of examples what control systems techniques can contribute to our understanding of these biological regulation systems. Although a great amount of physiology has yet to be learned about the relevant sub-systems involved before a definitive model can be established, it is the belief of Kadish and his group that the systems analyst can contribute to this effort by systematizing knowledge about the metabolic system in a consistent mathematical framework, and providing ideas tovthe physiologist about specific experiments suggested by model building, simulation, and analysis, and by determining analytically the consequences of conflicting theories concerning the operational dynamics of specific portions of the system.

Recognising that the system contains inherent nonlinearities such as saturation effects and threshold phenomena, the models were constructed from first order nonlinear differentiál equations. All the models are

simulated by digital computer and compared to actual data from controlled experiments in humans. Charette, Kadish and Srinavasan (1967) presented a report on some preliminary results in modelling of hormonal control of glucose metabolism. This work treated the glucose loop with two controlling hormones, insulin and glucagon (see Figure 12). The system is considered to be representative of a normal, 70 kg unstressed adult subject; sex hormones are neglected. Initial conditions are intended to depict a normal human plasma glucose regulator in a steady-state, ie. in metabolic and endocrine equilibrium, hence parameter dependencies are represented in terms of deviations from the nominal.

Hormone generation is modelled on the basis of the sequence, secretion, accumulation, and depletion. Physiologically, hormone secretion rates monotonically increase from nominal until saturation occurs. This action is modelled by the smooth and symmetric nonlinearity:

$$r = r_o + \alpha \tanh (\beta (e-e_o)) \qquad (14)$$

where r is the hormone secretion rate in (mass/unit time) and e is the stimulus. This particular analytic form was chosen with a view to simplifying the inverse or identification problem, which amounts to either a parameter optimization or two-point boundary value problem if and are assumed constant. The function 14 reproduces the so-called "hyperbolic" behaviour of first-order Michaelis-Menten rate kinetics and the so-called "sigmoid" behaviour of a series of such reactions with sufficient accuracy until more precise analytic forms are justified by physiological data. Such functions are used to model glycogenolysis, glycogenesis, insulin secretion and glucagon secretion. Following several investigators, the effect of hormones on the function (14) is modelled as a linear change to the saturation value of the non-linearity. For example:

Figure 12

The early glucose model of Charette et al. (1967) involving two hormones, insulin and glucagon.

$$r = r_o + \alpha \tanh (\beta (e-e_o))$$

$$r_o = (r_h + r_l)/2 \tag{15}$$

$$\alpha_o = (r_h - r_l)/2 \tag{16}$$

$$r_h = \gamma^h + r_{ho} \tag{17}$$

where h is the pertinent hormone concentration affecting the lumped rate kinetics (see equation 14), and γ is a constant. Accumulation and depletion are modelled by first-order kinetics, in which it is assumed that the degradation rate is proportional to the concentration, ie.

$$\dot{h} = -k_2 h + k_1 r \tag{18}$$

where k_2 has a value $(\underline{t}^{-1})$ and k_1 has a value $(\underline{v}^{-1})$. The parameters k_1 and k_2 have been measured by various techniques, eg. radioactive tracers, for several hormones, k_1 representing the circulatory volume and k_2 the degradation factor.

Consider the problem of establishing parameter values for the hormone model based on physiological data.

$$\dot{h} = k_2 h + k_1 (r + f_e)$$

$$r = r_o + \alpha \tanh (\beta (e-e_o)) \tag{19}$$

where f_e represents an exogenous input of the hormone h. Suppose the hormone under consideration is insulin, where (14) represents the generation and release of insulin from the pancreas into the plasma. Then we must identify k_1, k_2, r_o, α, β, and e_o. Theoretically the identification can be carried out in two steps. We will assume perfect observations to simplify the illustration.

If we administer $f_e(t)$, a known quantity of insulin intravenously,

the resulting hypoglycaemic state implies that r = 0 in (19) ie. the endogenous production of insulin is at or below its nominal value. Adjoining the parameters k_1 and k_2 to the differential equation (19) we obtain

$$\dot{h} = k_2 h + k_1 f_e$$
$$\dot{k}_1 = 0 \qquad (20)$$
$$\dot{k}_2 = 0$$

Given the input $f_e(t)$, $0 \leq t \leq T$ and three measurements of $h(t)$, $h(t_1)$, $i = 1,2,3$, these data and (20) constitute a multipoint boundary value problem which can be solved for k_1 and k_2 by the technique of quasi-linearisation. After each iteration the specific k_1 and k_2 obtained are substituted into (19) and the equation integrated to yield $\bar{h}(t)$, $0 \leq t \leq T$. Given some $\varepsilon > 0$, the process is repeated until some criterion such as,

$$\sum_{i=1}^{3} [h(t_i) - \bar{h}(t_i)]^2 < \varepsilon \qquad (21)$$

is satisfied.

Now assuming k_1 and k_2 fixed, and $f_e(t) \equiv 0$, a glucose input can be administered dictating $e(t)$, $0 \leq t \leq T$. Identifying r_i, e_o, α, and β can now theoretically be done in a similar manner with a minimum of six measurements on $h(t)$ by solving,

$$\dot{h} = -k_2 h + k_1 (r_o + \alpha \tanh (\beta (e-e_o))$$
$$\dot{r} = \alpha \beta \operatorname{sech}^2 (\beta (e-e_o)) \dot{e}$$
$$\dot{\alpha} = 0$$
$$\dot{\beta} = 0 \qquad (22)$$
$$\dot{r}_o = 0$$
$$\dot{e}_o = 0$$

and satisfying an expression such as (21).

Returning to the discussion of the first glucose regulation model derived by the authors (Figure 12), it can be more compactly written as follows :

$$\dot{c} = k_{19}c + K_{18}[F + f_{LO}(c,x_2) - f_{LU}(c,x_1,y_3) - f_R(c) - f_U(c,x_1) - GU)] \quad (23)$$

$$\dot{x}_1 = K_8x_1 + K_7 (I + f_1(c)) \quad (24)$$

$$\dot{x}_2 = K_{11}x_2 + K_{10}(G + f_2(c)) \quad (25)$$

where:

c	=	plasma glucose concentration
x_1	=	plasma insulin concentration
x_2	=	plasma glucagon concentration
y_3	=	liver glycogen content
F	=	intravenous glucose input rate
GU	=	central nervous system mean glucose utilisation rate
I	=	intravenous insulin input rate
G	=	intravenous glucagon input rate
$f_{LO}(c,x_2)$	=	liver glucose output rate
$f_{LU}(c,x_1,y_3)$	=	liver glucose uptake rate
$f_R(c)$	=	renal excretion rate
$f_U(c,x_1)$	=	peripheral tissue glucose utilisation rate dependent on insulin
K_{19}	=	hormone independent tissue utilisation rate coefficient.

Numerical values for the model parameters were obtained from results in the literature. Refinements were made by simulating experiments in which glucose or insulin were administered under

controlled conditions. All intravenous injection of glucose or insulin appears to the system as an impulse function. It was found that a finite duration step function was an adequate representation of such a test input. Simulations which correspond to tests ranging in duration from one to six hours in real time were performed.

The model more recently under consideration still focuses on glucose metabolism, but with four controlling hormones, insulin, glucagon, epinephrine, and growth hormone. The model for the metabolic system is shown in Figure 13, depicting the control of liver function, renal function, muscle and adipose tissue utilisation of glucose, by four controlling hormones through parametric feedback, in a one-compartment configuration. The model being used for the four-component hormonal controller is shown in Figure 14 and includes both proportional and rate sensitive control modes.

The signals for hormone action are taken to be glucose error and error rate. The model can also be represented as follows:

$$\tau_{18}c_1 + \dot{c}_1 = G_{18}\,(F(t-\tau_1) + f_{LO}(c_1,h_2,h_3) - f_{LU}(c_1,x_4,h_1) - f_r(c_1) - G_9c_1 - f_M(x_{11}) - f_L(x_{11},h_4)) \tag{26}$$

$$\dot{h}_1 = K_6h_1 + K_5\left\{K_1(h_3)\,[f_1(c_1) + f_2(\dot{c}_1)] + I\,(t-\tau_2)\right\} \tag{27}$$

$$\dot{h}_2 = -K_{11}h_2 + K_{10}\,[f_3(c_1) + G\,(t-\tau_3)] \tag{28}$$

$$\dot{h}_3 = -K_{21}h_3 + K_{20}[f_4(c_1) + f_5\,(c_1) + E(t-\tau_4)] \tag{29}$$

$$\dot{h}_4 = -K_{31}h_4 + K_{30}[f_6(c_1) + S(t-\tau_5)] \tag{30}$$

where:

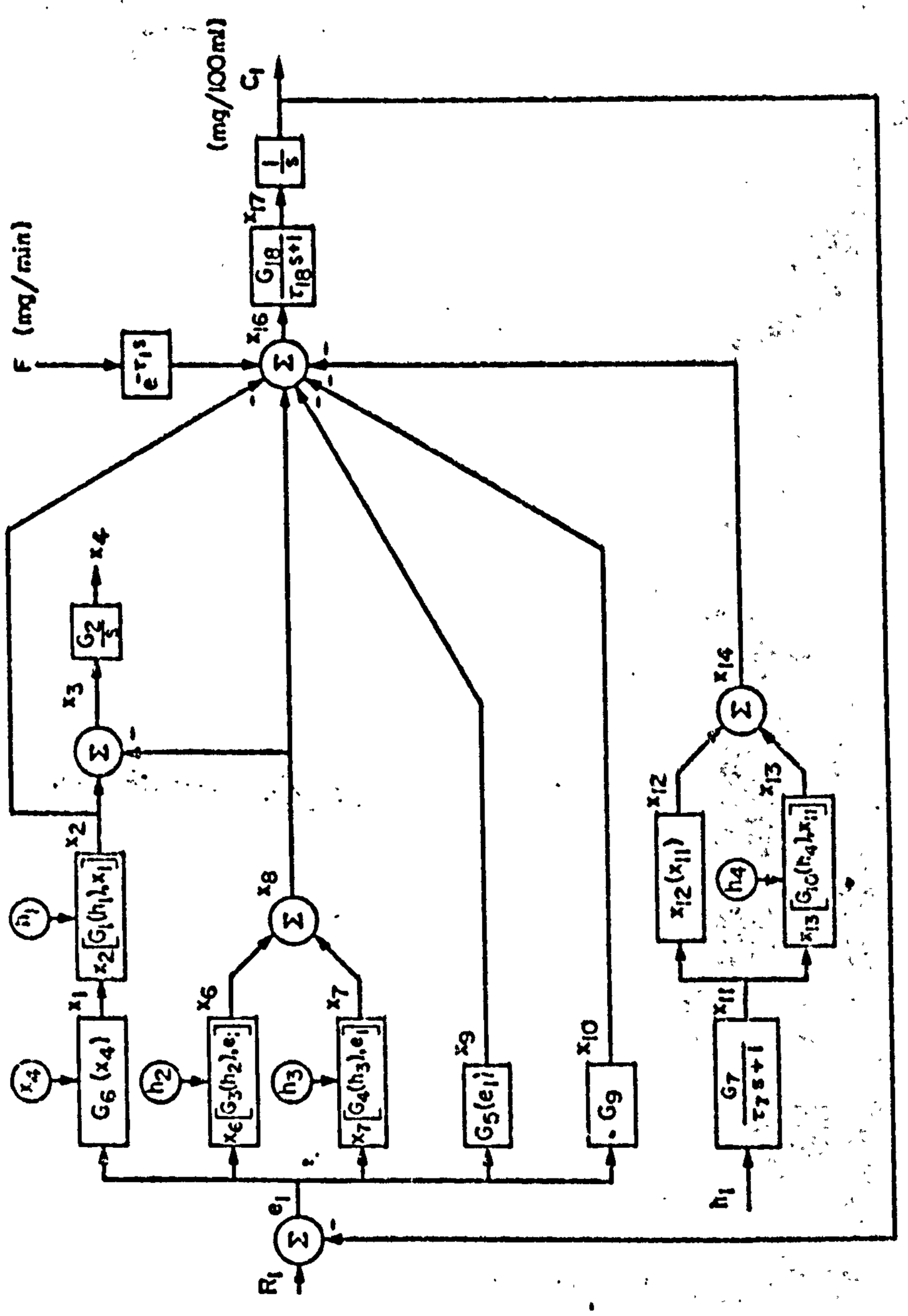


Figure 13. The glucose metabolic system incorporated in the model of Charette, Kadish and Sridhar (1969).

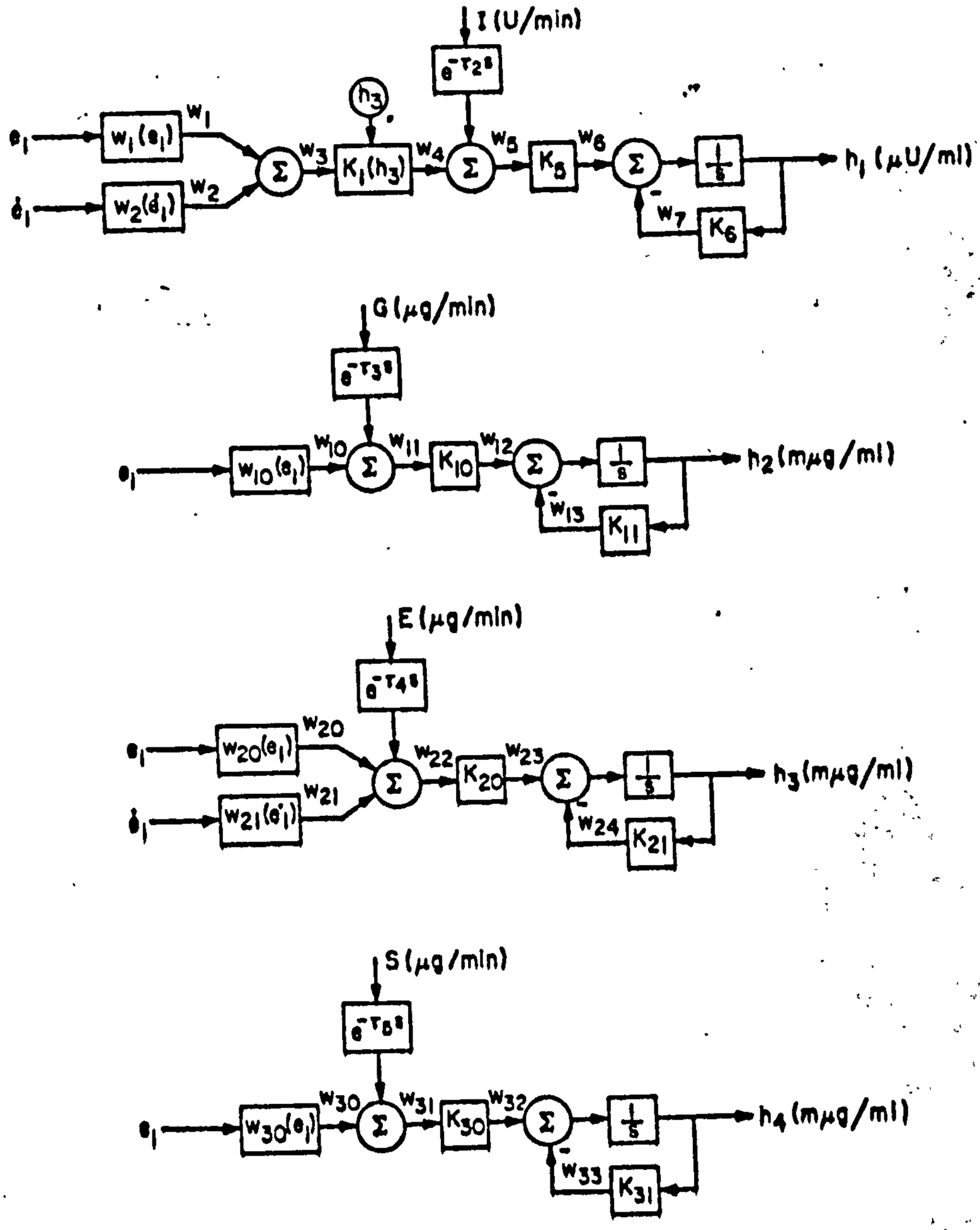


Figure 14. The hormone controllers incorporated in the model of Charette, Kadish and Sridhar (1969).

$$x_{11}(t) = \frac{G_7}{7}\int_0^t e^{-(t-\tau)/\tau_7} \; h_1(\tau)d\tau \qquad (31)$$

The parameters of this model were adjusted to simulate the results of experiments.

This group claims (Charette, Kadish & Sridhar 1969) that their model was successfully used to simulate the results from two experiments, one involving a short intravenous infusion of glucose over three minutes, and the second, in which five units of insulin were injected intravenously followed by 1 mg of glucagon 60 minutes later. However, no confirmatory evidence of these simulations was presented in the review.

However, Srinivasan, Kadish and Sridhar (1970) presented another model based upon the earlier model of Charette, Kadish and Sridhar (1969) which probably represents the most sophisticated attempt to date, though its complexity is in no way a guarantee as to the correctness of the model. Non-linearities such as thresholds and saturation effects were generated with a hyperbolic tangent function with the following characteristics: its lower and upper saturation values and the steepness of the rise from one to the other were adjustable using three parameters. The upper saturation value, when postulated to be parameter dependent, was linearly dependent. The model included the substrates, glucose and free-fatty acids, and the hormones insulin, glucacon, adrenaline and growth hormone. The glucose distribution space included the plasma and interstitial compartments. Gluconeogenesis was assumed to be brought about by raised free-fatty acid concentration, and its saturation level was regulated by the plasma glucagon and adrenaline concentrations. Hepatic glycogenolysis was assumed to be stimulated by a fall of the glucose concentration below the resting plasma glucose concentration. The saturation value of this process was also regulated by the same two hormones. Glycogen synthesis

in the liver was set to respond to the deviation of the plasma glucose concentration above the fasting level. The saturation of this process was regulated by the plasma insulin concentration.

Insulin secretion was responsive to both the change in plasma glucose concentration and in the rate of change; adrenaline at raised concentrations inhibits this secretion. Insulin distribution included plasma and interstitial fluid compartments. Glucagon secretion was inversely related to the plasma glucose level. Adrenaline secretion was assumed to be responsive to the plasma glucose concentration and its rate of change. A single distribution volume was assigned to adrenaline. Growth hormone secretion was assumed to respond to the plasma glucose concentration.

The incorporation of all these variables made this a very complex model, with the glucose portion containing forty parameters alone. The model was validated with data obtained from intravenous glucose and insulin injections in two subjects. The plasma glucose, insulin and free-fatty acid responses were fitted to the model. These results are apparently the same as those mentioned in the earlier paper. However, a number of additional tests were performed with the model without using experimental data, to show that expected conformations or trends in the responses could be produced. These tests were intravenous boli of glucagon,adrenaline and growth hormone.

Although the model does not reproduce adequately the finer details of the actual responses, as represented by the laboratory data, the authors feel that their limited success was vindication of their approach. Yet a further description of the use of this model was given by Campfield (1973), who emphasised the correspondence of the model with the real physiological system. He also considered the principal deficiencies of the model were the omission of the corticosteroids, catecholamines and the autonomic nervous system. The model was claimed to simulate various forms of hypoglycaemia, but no confirmatory evidence is presented.

Recently Foster et al. (1973) have described a model incorporating two substrates, glucose and free-fatty acids, and two hormones, insulin and glucagon. The non-linearities of the model are generated by table functions (function generators) which produce piece-wise linear curves. The glucose pool includes a mixing compartment, muscle and the liver. The rate of change of glucose concentration is assumed to be governed by both insulin and glucagon acting in an antagonistic manner, whereas that of free-fatty acids is affected by insulin only. Nervous system and red blood cell glucose consumptions and the kidney threshold for glucose were included.

The secretion of insulin was related to the glucose level in the glucose space, to its rate of change, and to the FFA level. The secretion of glucagon was related to the glucose level. Effects on adrenaline were incorporated as enhanced glucose output by liver, increased FFA release and depressed insulin secretion, all following hypoglycaemia as a stimulus to epinephrine secretion.

The model was validated with oral and IV glucose tolerance tests, and studies were performed to characterise the diabetic state that the normal glucose tolerance test was dominated by changes in insulin secretion dependent on changes in the level of glucose. The model was very insensitive to changes in the size of the glucose space (50% increase and 20% decrease had almost no effect).

This paper by Foster and his co-workers presents a non-linear complex model of glucose regulation in man that can apparently successfully simulate experimental results even when certain of the criteria of the model do not fit real conditions. On the other hand, it represents a failure of the model to offer insight into the physiological processes because the model behaviour can be satisfactory in the absence of reasonable degree of isomorphism to the physiology. The model was developed as part of a project to construct an "artificial beta cell" for use in the treatment of diabetes. This work

presents an interesting and important attempt to reconcile a clinical engineering approach and a physiological one. Certain defects of the model, as initially conceived in simulating observed behaviour, led to its modification. This awareness of the ability of modelling to suggest new experiments, led the author to present a revised theory of the nature of control of insulin release, though the evidence is slight.

The inherent weakness of the models of Srinivasan et al. (1970) and Foster et al. (1973) is that whilst structurally isomorphic they are not so functionally. Both groups have suggested that their system performance is adequate but as with so many other modelling attempts they are conformational in nature. For instance neither is able to reproduce the oscillatory behaviour of the plasma glucose metabolic system which has been demonstrated in vivo (Iberall, Ehrenberg & Cardon 1968). A possible reason for this is that the representations chosen for the most significant parts of the system do not reflect the most important non-linearities of the sub-systems. It is therefore obviously necessary to identify and define the dynamic characteristics of the most relevant unit processes of the system, namely the enzymes involved.

Several factors are involved in the regulation of enzyme activity. In the work described below only the direct modulation of enzyme activity is considered; the entire area of genetic control, where the amount of enzyme directly synthesised can be regulated by induction, repression and derepression is excluded. Direct control can occur in one of two ways: either through the catalytic mechanism or by coupling the catalytic mechanism with other processes.

The simplest form of control, and probably the most primitive, of the catalytic mechanism is the saturation type of kinetics normally governing enzyme reactions. As substrate concentrations increase, the rate of reaction increases until a limiting value is attained; as product accumulates

the reaction rate is decreased by product inhibition. In this process the substrate and products act as "on" and "off" switches and the concentration of metabolites can regulate the catalytic rate. A similar control occurs with coenzymes, though the concentrations of coenzymes are normally not sufficient to saturate all coenzyme requiring enzymes, so that in turn the relative coenzyme affinity and enzyme concentrations provide some regulation.

Enzymes with a single ligand binding site give binding isotherms that are always hyperbolic. However, with enzymes that possess multiple binding sites, usually implying multiple subunits, nonhyperbolic binding isotherms are possible. In cases where binding is enhanced as successive ligands bind, sigmoidal isotherms are obtained, and this [15] found even where multiple subunits are formed from checmially identical subunits. A sigmoidal binding curve has a region where the degree of saturation is more sensitive to the substrate concentration than the hyperbolic isotherm; therefore the enzymatic reaction rate can be closely regulated by the substrate concentration. Contrariwise the binding may be depressed as successive ligands bind, providing an alternative control process.

Enzyme activity may also be controlled by coupling the catalytic reaction to other processes which usually involve regulation by ligands not participating directly in the reaction and often structurally unrelated to the substrates. Typical examples are feedback inhibition, where the metabolic end product of a pathway controls its own production by inhibiting the activity of an early enzyme in the pathway (Umbarger 1961); precursor activation, where an early metabolite activates a later enzyme in a metabolic sequence (Leloir and Goldenberg 1960); polymerisation - depolymerisation triggered by protein concentration changes or ligand binding (Frieden 1968): energy link control by adenylates (Atkinson 1969) and hormone control, where the details of regulation are as yet not well understood, although cyclic -3',5'- AMP is thought to have an important role (Robison, Butcher and Sutherland 1971).

Multienzyme complexes provide a further type of enzyme regulation. By combining two or more enzymes into a single molecular aggregate the activity of the individual enzymes can be modulated through protein - protein interaction. Also catalytic features can arise, such as processing a substrate through several enzymes without dissociation.

The membrane also provides a potential basis for modulating enzyme activity, not only by localisation, but by altering the enzymatic reaction through protein-lipid interaction. However, such systems are difficult to study as isolation of the membrane invariably disrupts the homogeneity of the system.

Thus, any approach to understanding regulatory mechanisms must involve structural, thermodynamic and dynamic considerations. In this work the emphasis is upon the dynamics of control together with an attempt to make use of structural data. In the next chapter details of the mathematical model based upon these concepts is described.

CHAPTER 5

DESCRIPTION OF THE NEW MODEL

The development of the model to describe glucose dynamics in the mode detailed here, that is in terms of enzyme kinetics,requires knowledge of:

i) the rate determining steps in the reactions;

ii) the kinetics of each reaction;

iii) the factors regulating the enzyme involved;

iv) and the kinetics of flux (rates).

The reactions considered in the model are depicted in Figure 17 and these biochemical features and certain physiological assumptions were incorporated into the model.

I. Biochemical Features of the Model

In the liver there is no permeability barrier to glucose - the hepatocyte membrane being freely permeable. Thus the membrane is neglected in the model and the glucose concentration assumed to be equal in plasma and liver water (Cahill et al. 1958; Gey 1956). This assumption would appear to be valid, for [14C]glucose rapidly equilibrates with the intracellular pool and the rate of equilibration is independent of the direction of glucose flux. Thus the rate-limiting step in glucose utilization by hepatic tissue is determined by the glucose phosphorylation rate, rather than the rate of entry into the cell (Cahill et al. 1959).

The rate of glucose-6-phosphate production by glucogenesis, is fixed at 1×10^{-4}M per minute, which is near the rate obtained for incorporation of labelled pyruvate into glucose and glycogen in experiments performed by Ashmore et al. (1956) using liver slices. It is further assumed that in the steady-state this constant rate is the resultant for the pathways

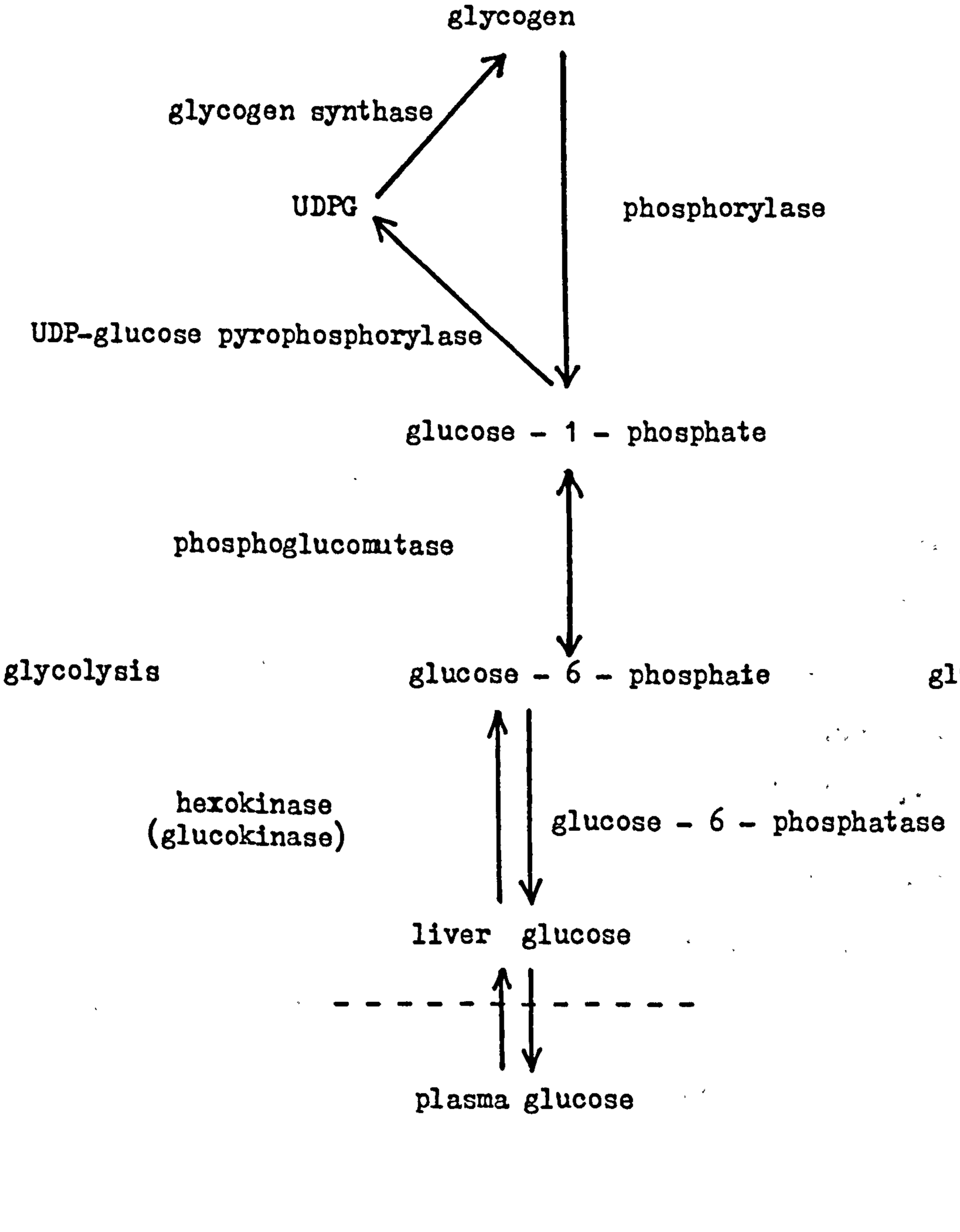


Figure 15 The enzymatic reactions involved in the mathematical model.

that yield glucose-6-phosphate and those that utilize this compound.

It is also assumed that the reactions involved in the branching and debranching of glycogen, can be ignored as their processes occur only at the periphery of the molecule of the glycogen polymer (Stetten and Stetten 1960).

The reactions involving conversion of UDP-glucose to UDP-galactose and UDP-glucuronic acid, are also ignored, as they are minor and are not directly concerned with the storage or dissimilation of glycogen.

Enzyme mechanisms

Enzyme reaction rates have been specified in terms of data obtained from *in vitro* experiments described in the literature. The data has been interpreted in terms of individual reaction mechanisms, using Briggs-Haldane kinetic theory (Briggs and Haldane 1925); that is, assuming the practical and approximate steady-state. This steady-state is assumed to be of such a relative and temporary nature (Chance 1943), that it can be directly applied to transient states (see Appendix I).

Thus, enzymatic rate equations, or more correctly enzyme kinetic equations, were derived directly from the reaction mechanisms for the enzyme substrate (Cleland 1963).

The enzymatic mechanisms and the appropriate rate equations appear in Table II.

Glucokinase (E.C.2.7.1.12) and Hexokinase (E.C.2.7.1.1)

The rate equation for glucokinase is a modified Briggs-Haldane equation where V_1 is the maximum velocity, and the kinetic constant of the substrate was determined in the presence of a high concentration of the other substrate. The empirical equation is consistent with the data of Salas et al. (1965) with inhibition by ADP omitted. This latter inhibition is poorly understood. In the normal subject

1) Glucokinase

$$ATP + Glucose + E_1 \rightarrow Glucose\text{-}6\text{-}phosphate + ADP + E_1$$

$$V_1 = \frac{V_1\, G}{K_G^1 + G\,(K_{ATP}^1 / ATP + 1}$$

2) Glucose-6-phosphatase

$$Glucose\text{-}6\text{-}phosphate + H_2O + E_2 \rightarrow Glucose + Inorganic\ phosphate + E_2$$

$$V_2 = \frac{V_2 G6P}{K_{G6P}^2 + G6P}$$

3) Phosphoglucomutase

$$Glucose\text{-}6\text{-}phosphate + E_3 - P_i \rightleftharpoons E_3 + G1, 6\ di\text{-}P \rightleftharpoons Glucose\text{-}1\text{-}phosphate + E_3 - P_i$$

$$V_3 = \frac{(V_{31} \cdot K_{G1P}^3 \cdot G6P) - (V_{32} K_{G6P}^3 \cdot G1P)}{}$$

$$K_{eq}^3 = \frac{G6P}{G1P} = \frac{V_{32}\, K_{G6P}^3}{V_{31}\, K_{G1P}^3}$$

4) Glucose-1-phosphate uridylytransferase

$$Uridine\ triphosphate + Glucose\text{-}1\text{-}phosphate + E_4 \rightleftharpoons Uridine\ diphosphate\ glucose + PP_i + E_4$$

$$V_4 = \frac{V_{41}\, V_{42}\, (UTP.G1P) - (PP_i\, UDPG/K_{eq}^4)}{(K_{G1P}^4 \cdot V_{42} \cdot UTP) + (K_{UDPG}^4 \cdot V_{41} \cdot PP/K_{eq}^4) + V_{42}\,(K_{UTP}^4 + UTP).\ G1P + V_{41}\,(K_{pp} + PP).\ UDPG/K_{eq}^4}$$

$$K_{eq}^4 = \frac{PP.UDPG}{UTP.G1P} = \frac{V_{41}^2}{V_{42}} \quad \frac{K_{pp}^4\ K_{UDPG}^4}{K_{UTP}^4\ K_{G1P}^4}$$

Table II Enzyme mechanisms and rate equations [cont'd]

5) <u>Glucogen synthase</u>

$$\text{Glucose-6-phosphate} + E_5 \overset{1}{\rightleftharpoons} C_{51}$$

$$C_{51} + \text{Uridine diphosphate glucose} \overset{2}{\rightleftharpoons} C_{52} \overset{3}{\longrightarrow} C_{51} + \text{products}$$

$$E_5 + \text{Uridine diphosphate glucose} \overset{4}{\rightleftharpoons} C_{53} \overset{5}{\longrightarrow} E_5 + \text{products}$$

$$V_5 = V_5 \frac{G6P + (k_5/k_3)([K_{51} \cdot K_{52}]/K_{53})\ UDPG}{K_{52}(G6P + K_{51}) + ([K_{51}\ K_{52}/K_{53}] + G6P)\ UDPG}$$

6) <u>Phosphorylase</u>

$$\text{Adenosine monophosphate} + E_6 \overset{1}{\rightleftharpoons} C_{61}$$

$$\text{Glucose-6-phosphate} + E_6 \overset{2}{\rightleftharpoons} C_{62}$$

$$C_{61} + P \overset{3}{\rightleftharpoons} C_{63} \overset{4}{\rightleftharpoons} C_{64} \overset{5}{\rightleftharpoons} C_{61} + G1P$$

$$V_6 = \frac{(V_{61} \cdot K^6_{G1P} \cdot P) - (V_{62} \cdot K^6_p \cdot G1P)}{K^6_{G1P} \cdot P + K^6_p \cdot G1P + K^6_p \cdot K^6_{G1P}\ (K^6_{AMP}/AMP\ ([G6P/K^6_{G6P}] + 1) + 1)}$$

$$K^6_{eq} = \frac{P}{G\text{-}1\text{-}P} = \frac{V_{62}\ K^6_P}{V_{61}\ K^6_{G\text{-}1\text{-}P}}$$

glucokinase accounts for about 80% of the phosphorylating capacity of hepatic tissue (Salas et al. 1965; Vinuela, Salas and Sols 1963; Salas, Vinuela and Sols 1963; Sharma, Rajani and Weinhouse 1963). The contribution of hexokinase accounts for the remaining activity.

Glucose-6-phosphatase (E.C.3.1.3.9)

The mechanism proposed by Hass and Byrne (1960) for glucose-6-phosphatase has been used; the rate equation is a typical Briggs-Haldane equation. Any slight inhibition by Glucose and inorganic phosphate is neglected (Hass and Byrne 1960a; 1960b).

Phosphoglucomutase (E.C.2.7.5.1)

The mechanism for rabbit muscle phosphoglucomutase as suggested by Najjar and Pullman (1954) has been incorporated. Kinetic constants for the rate equation were calculated using the data from Bodansky (1961) with initial conditions for the reaction including the presence of excess cofactor. This yielded an equilibrium constant of 12.7.

Glucose-1-phosphate uridylytran erase (E.C.2.7.7.9; UDP-glucose pyrophosphorylase)

The mechanism incorporated for UDP glucose pyrophosphorylase was that proposed by Munch-Petersen (1955) for yeast. The equilibrium constant of unity as suggested by Kornfeld (1965) is accepted. The kinetic constant for one substrate was determined with excess substrate but in the absence of products. The dissociation constants for UTP and UDP-glucose are assumed to be large and are not incorporated.

Glycogen synthase (E.C.2.4.1.11; UDP-glucose-glycogen glucosyl transferase)

The mechanism used assumes that glucose-6-phosphate affects the kinetic content for UDP-glucose but not the maximum velocity constant (Kornfeld and Brown 1962; Traut and Lipmann 1963; Rosell-Perez and Larner 1964) which implies that glucose-6-phosphate combines initially

with the enzyme. The liver enzyme is strongly dependent on glucose-6-phosphate (Leloir and Goldenberg 1960; Steiner and King 1964). However, the possibility of catalysis without is included to simulate conditions for varying concentrations of glucose-6-phosphate. The mechanism assumes excess glycogen and no effect of UDP.

Glycogen phosphorylase (E.C.2.4.1.1)

The properties of hepatic glycogen phosphorylase have not been so closely studied as those of the muscle enzyme, and it is not very clear how the inorganic phosphate concentration varies with glucose concentration. Therefore, the rate of phosphorylase is maintained near constant as an approximation. To account for changing phosphate concentration a reaction mechanism based on data for muscle phosphorylase b is used (Helmreich and Cori 1964; Morgan and Parmeggiani 1964).

This assumes dependence upon 5'-AMP, inhibition by glucose-6-phosphate and excess glycogen. The observation of Helmreich and Cori (1964) that 5'-AMP does not affect the V_{max} implies that this activator combines with enzyme before substrates.

The suggested mechanism predicts that the observed kinetic constants for P_i, glucose-1-phosphate, glucose-6-phosphate and 5'-AMP may vary with changing concentrations of substrate, activator or inhibitor. However, true kinetic constants are obtainable which are independent of the concentration of any substance (see Appendix II for the method of calculating these constants). The equilibrium constant calculated from these data is 3.1 at pH 7.5.

With near constant phosphorylase activity the model is not dependent on this mechanism of reaction or rate equation except when the inorganic phosphate concentration is varying/changing. Small changes that occur in phosphorylase activity are the result of slight glucose-6-phosphate inhibition, for phosphate and 5'-AMP concentrations

are constant and the low glucose-1-phosphate does not produce a significant back reaction. Dependence of phosphorylase on 5'-AMP and glucose-6-phosphate inhibition are a means to simulate slight changes in enzymatic activity.

The derived input constants used in the present model are given in Table III.

The values were obtained from the literature with the following exceptions:

i) Constant concentrations of UTP and PP_i were used to simplify calculation. Reported UTP and PP_i concentrations together with a UDP-glucose concentration of 3×10^{-4} mol (Hornbrook, Burch and Lowry 1965) and an approximate physiological glucose-1-phosphate concentration of 4×10^{-6} mol predict net UTP production by UDP-glucose pyrophosphorylase.

This would suggest that the PP_i concentration is too high (or UTP concentration is too low) as the enzyme catalyses UDP-glucose production as part of the glycogen synthesis system.

ii) In order to have adequate synthase activity, yet assuming a strong dependence on low glucose-6-phosphate concentrations (Hornbrook, Burch and Lowry 1965) a small dissociation constant for glucose-6-phosphate was chosen. However, the precise determination of this constant is difficult.

iii) An alteration in the maximal velocities of phosphorylase is necessary to ensure a physiological balance between the phosphorylases and glycogen synthase. The V_{max} of the two enzymes is similar but the very low glucose-6-phosphate concentration limits the glycogen synthase activity markedly. This alteration in phosphorylase activities high-

Kinetic data	Value	Source of value
V_1	2.5×10^{-3}	Salas et al. 1965; Vinuela, Salas and Sols 1963.
K_G^1	2.27×10^{-2}	Walker 1963.
K_{ATP}^1	3.00×10^{-4}	Walker 1963.
V_2	1.20×10^{-2}	Weber and Cantero 1955; Dickens, Glock and McLean 1959.
K_{G6P}^2	2.00×10^{-3}	Hass and Byrne 1960; Segal and Washko 1959.
V_{31}	1.04×10^{-2}	Vollar-Palasi and Larner 1960.
V_{32}	3.77×10^{-2}	Calculated from K_{eq}^3.
K_{G6P}^3	8.65×10^{-5}	Bodansky 1961.
K_{G1P}^3	2.46×10^{-5}	Bodansky 1961.
V_{41}	1.06	Calculated from K_{eq}^4.
V_{42}	5.90×10^{-2}	Villar-Palasiand Larner 1960.
K_{UTP}^4	3.00×10^{-3}	Breckenridge et al. 1961.
K_{G1P}^4	1.00×10^{-3}	Breckenridge et al. 1961.
K_{UDPG}^4	9.0×10^{-5}	Kornfeld 1965.
K_{PP}^4	1.54×10^{-4}	Breckenridge et al. 1961.
V_5	3.12×10^{-3}	Leloir et al. 1959.
K_{51}	1.5×10^{-4}	Leloir and Goldemberg 1960.
K_{52}	4.5×10^{-4}	Leloir and Goldemberg 1960.
K_{53}		
$K_{54}(K5/K3)$	0	

Table III. Kinetic data and constant concentrations. [cont'd]

Kinetic data	Value	Source of value
V_{61}	1.25×10^{-3}	Villar-Palasi and Larner 1960.
V_{62}	3.12×10^{-2}	Calculated from K^6_{eq}
K^6_P	1.00×10^{-3}	Helmreich and Cori 1964.
K^6_{G1P}	8.00×10^{-4}	Helmreich and Cori 1964.
K^6_{AMP}	3.60×10^{-4}	Helmreich and Cori 1964.
K^6_{G6P}	2.50×10^{-4}	Morgan and Parneggiani 1964.
Gluconeogenesis	1.00×10^{-4}	Ashmore et al. 1956
Constant Concentrations		
ATP	2.45×10^{-3}	Heldt 1963.
UTP	1.80×10^{-3}	Heldt 1963.
PP_i	1.60×10^{-5}	Heldt 1963.
P_i	3.00×10^{-3}	Heldt 1963.
AMP	1.30×10^{-4}	Heldt 1963.

Note The units of the velocity constants and for gluconeogenesis are moles litre^{-1} min^{-1}, those for the kinetic constants and concentrations moles litre^{-1}.

lights the inadequacy of the phosphorylase scheme, but the complexity of which could be overwhelming, if for instance the interconversion of active and inactive forms was introduced. The activity of the enzyme was maintained near constant in the model.

Alteration of the maximal velocities is the simplest way of limiting the activity but alteration of the kinetic constants or concentrations of reactants would be as effective. In this study variation of the phosphate concentration is thought to be important.

If changes in K_{51} , V_{61} and V_{62} are not made glycogen breakdown will exceed its synthesis and the rates would only be equal at unrealistic levels.

II. Further assumptions concerning model function

i) Plasma glucose concentrations below nominal ie. resting or fasting level (negative glucose error) will bring about glycogenolysis while concentrations above nominal (positive glucose error) lead to glycogenesis. The former process is facilitated by glucagon and adrenaline, and the latter process by insulin.

ii) Gluconeogenesis is facilitated by glucagon and adrenaline.

iii) Glycogen storage in the liver is limited by a saturation concentration of approximately 25 μmol of glucose as glycogen per gram of liver. That is, the glycogen content of the average 1500 g liver is about 70 g. Glycogenesis is cut off when the glycogen stores reach their capacity.

iv) There is no renal excretion of glucose below an assigned threshold value of plasma glucose concentration.

v) The utilisation of glucose by cerebral tissue and the erythrocytes is at a constant rate independent of the plasma glucose concentration.

vi) The rate of glucose uptake by peripheral tissues is a function of insulin concentration, in the interstitial tissues, which facilitates the active transport of glucose across the cell membranes.

vii) The dynamics of glucose distribution are determined by two compartments: the extracellular fluid, including plasma, erythrocyte and liver water volumes, and the intracellular fluid volume. This lumping of plasma, hepatic and erythrocyte glucose is justified because of the rapid equilibration of glucose concentration in liver and erythrocytes with that of the plasma.

viii) The hormones included in the model are insulin, glucagon and adrenaline. All are involved in homeostasis.

ix) Glucose error stimulates the secretion of these hormones. Positive glucose error stimulates insulin secretion, negative error glucagon and adrenaline secretion.

x) The insulin and adrenalin secretion rate is stimulated by a high glucose error rate.

xi) Adrenaline inhibits insulin secretion.

xii) Hormones are distributed and degraded in a linear fashion.

III. Formulation of the Mathematical Model

It was realised that it would not be feasible to produce a realistic model of the metabolic system shown in Figure II in terms of all the unit processes as there are too many gaps in our knowledge.

The initial attempt to produce a mathematical model was based on the reduced model shown in Figure 16.

All the systems studied were considered as an assembly of interacting compartments and a mass balance equation written for each. The rate of mass

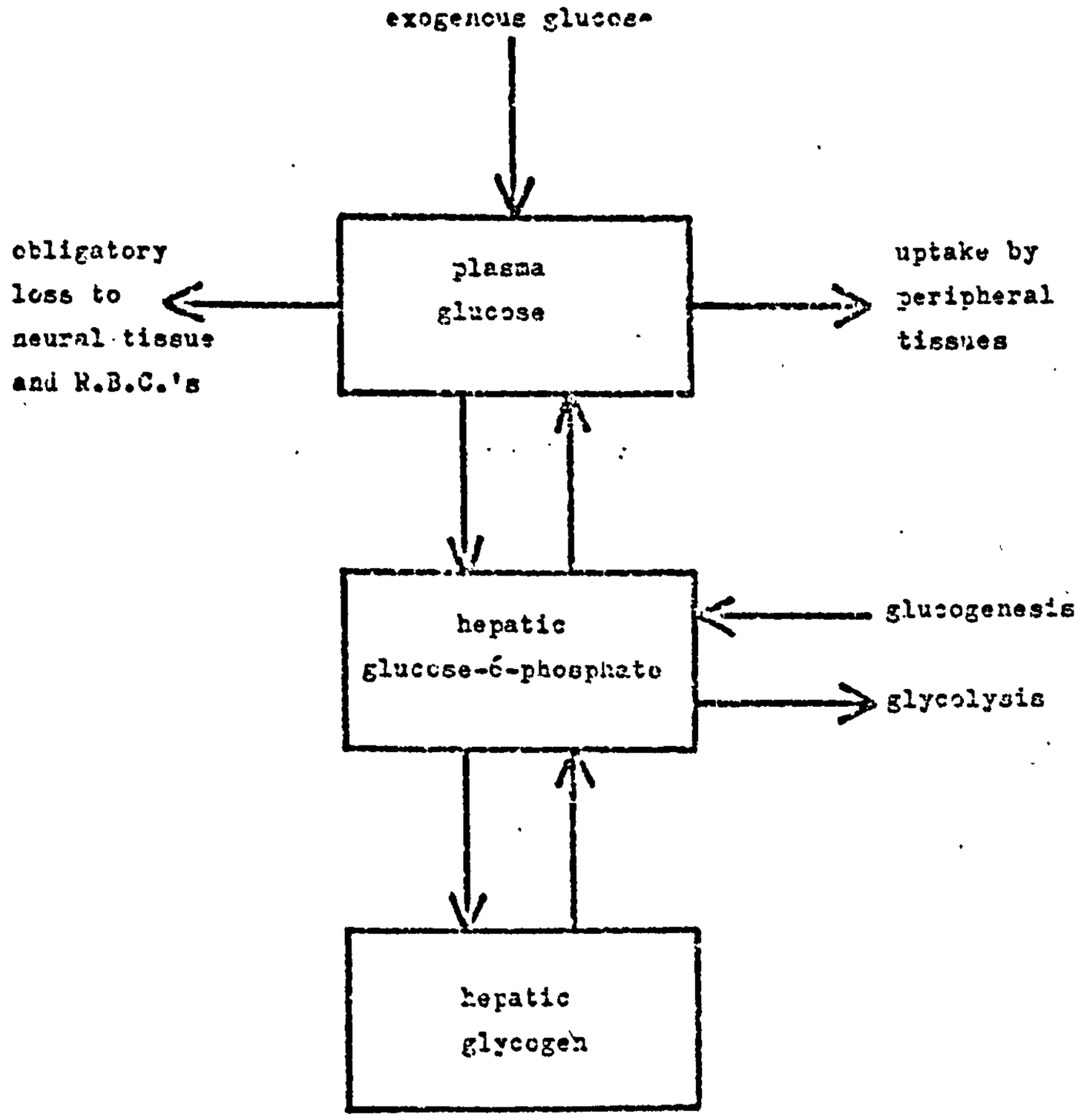


Figure 16. The reduced functional model of liver glucose metabolism.

transfer from compartment to compartment would be dependent upon the quantity of substance in the source compartment and a function of the kinetics of the enzyme reaction involved.

The following model notation was adopted:

Variables

Xn = concentration of substance in the nth compartment

Kmn = rate constant associated with transport of material from compartment m to compartment n (min^{-1})

fmn(Xm) = non-linear function of Xm describing rate of change of concentration during transport from m to n

K = constant rate of change of concentration (mol/min)

A primed variable indicates a threshold value.

Subscripts (indicating location of substance)

1 - plasma glucose

2 - liver glucose -6- phosphate

3 - triose phosphate

4 - glycogen

5 - pyruvate

6 - insulin

7 - glucagon

8 - adrenaline

9 - liver triglyceride

0 - represents basal secretion rate

a - oxidation

b - brain glucose compartment

c - lactate

I - is associated with insulin

J - is associated with glucagon

E - is associated with adrenalin.

Using this notation the following mathematical description of the model was obtained:

Plasma glucose compartment

$$\frac{dX_1}{dt} = \text{exogenous glucose dose rate} + k_{21} X_2 - k_{1b} - f_{12}(X_1)$$

Glucose -6- phosphate compartment

$$\frac{dX_2}{dt} = k_{32} X_3 - k_{23} X_2 - k_{21} X_2 + f_{12}(X_1) - \begin{cases} f_{24}(X_2) & [\text{for } X_6 > X_6'] \\ 0 & [\text{for } X_6 > X_6'] \end{cases}$$

$$+ \begin{cases} f_{42}(X_2) & [\text{for } X_7 > X_7' \text{ OR } X_8 > X_8'] \\ 0 & [\text{for } X_7 \leqslant X_7' \text{ AND } X_8 \leqslant X_8'] \end{cases}$$

Triose phosphate compartment

$$\frac{dX_2}{dt} = k_{53} X_5 - k_{35} X_3 - k_{32} X_3 + k_{23} X_2 - \begin{cases} k_{39(1)} X_3 & [\text{for } X_6 \leqslant X_6'] \\ k_{39(2)} X_3 & [\text{for } X_6 > X_6'] \end{cases}$$

Glycogen compartment

$$\frac{dX_4}{dt} = \begin{cases} f_{24}(X_2) & [\text{for } X_6 > X_6'] \\ 0 & (\text{for } X_6 \leqslant X_6'] \end{cases} - \begin{cases} f_{42}(X_2) & [\text{for } X_7 > X_7' \text{ OR } X_8 > X_8'] \\ 0 & [\text{for } X_7 \leqslant X_7' \text{ AND } X_8 \leqslant X_8'] \end{cases}$$

Pyruvate compartment

$$\frac{dX_5}{dt} = k_{33} X_3 - k_{53} X_5 + K_{05} - k_{5a} X_5$$

Insulin

$$\frac{dX_6}{dt} = K_{Io} + \text{exogenous insulin dose rate} + \begin{cases} K_{\underline{r}(1)} (X_1 - X_1') & [\text{for } X_1 > X_1'] \\ 0 & [\text{for } X_1 \leqslant X_1'] \end{cases}$$

$$+ \begin{cases} k_{I(2)} \left(\frac{dX_1}{dt}\right) & \left[\text{for } \frac{dX_1}{dt} > 0\right] \\ 0 & \left[\text{for } \frac{dX}{dt} \leqslant 0\right] \end{cases} - f_{\underline{r}}(X_6)$$

Glucagon

$$\frac{dX_7}{dt} = K_{J_o} + \text{exogenous glucagon dose rate} + \begin{cases} K_{J(1)}(X_1 - X_1') & [\text{for } X_1 > X_1'] \\ 0 & [\text{for } X_1 \leqslant X_1'] \end{cases}$$

$$+ \begin{cases} K_{J(2)} \left(\frac{dX_1}{at}\right) & \left[\text{for } \frac{dX_1}{at} > 0\right] \\ 0 & \left[\text{for } \frac{dX_1}{at} \leqslant 0\right] \end{cases} - k_{J(3)} X_7$$

Adrenaline

$$\frac{dX_8}{at} = K_{E_o} + \text{exogenous adrenaline dose rate} + \begin{cases} K_{E(1)} (X_1' - X_1) & [\text{for } X_1 < X_1'] \\ 0 & [\text{for } X_1 \geqslant X_1'] \end{cases}$$

$$+ \begin{cases} k_{E(2)} \left(\frac{-dX_1}{dt}\right) & \left[\text{for } \frac{dX_1}{at} < 0\right] \\ 0 & \left[\text{for } \frac{dX_1}{at} \geqslant 0\right] \end{cases} - k_{E(3)} X_8$$

Details of the non-linear functions, values for rate constants, basal secretion rates and physiological threshold values incorporated in the model are given in Table IV..

The method of calculation of these parameters and functions is as follows:

$$f_{12}(X_p) = \frac{12.0\ G_p}{22.7 + 1150.0\ G_p} \quad \text{mol min}^{-1} \quad \text{(London 1966).}$$

Substrates have the units of molar concentration

$$k_{16} = 80 \text{ mg min}^{-1} \times \frac{1}{18 \times 3.2 \times 10} \frac{\text{m mol}}{\text{mg } 1} = 0.000139 \text{ mol min}^{-1}$$

A plasma volume of 3,21 is assumed.

k_{21} = 0.0015 = 7.5 min^{-1} from graph of glucose-6-phosphatase (Fig.17)

$$k_{32} = \frac{20.0 \text{ mmol min}}{17.0 \text{ mmol}} = 1177.0 \text{ min}^{-1}$$

$$k_{23} = \frac{5.0 \text{ mmol min}}{217.0 \text{ mmol}} = 23.1 \text{ min}^{-1}$$

$$f_{24}(X_2) = \frac{1.25 X_2}{0.10722 + 1848.3 X_2} \text{ mol min}^{-1}$$

$$f_{42}(X_2) = \frac{0.03 - 31.2 X}{32.0 - 1000.0 X_2 + \frac{11.1}{4000.0 X_2 + 1.0}} \text{ mol min}^{-1}$$

$$k_{53} = \frac{13.0 \text{ mmol min}^{-1}}{65.0 \mu\text{mol}} = 200.0 \text{ min}^{-1}$$

$$k_{35} = \frac{50.0 \text{ mmol min}^{-1}}{17.0 \mu\text{mol}} = 2950.0 \text{ min}^{-1}$$

$$f_{eo}(N_e) = \frac{6.95 N_e}{0.000178 + N_e} \text{ mol min}^{-1}$$

k_{65} = 0.23 mol min^{-1}

$$k_{5a} = \frac{220.0 \mu\text{mol min}^{-1}}{65.0 \mu\text{mol}} = 3.39 \text{ min}^{-1}$$

k_{10} = 4.0 m U min^{-1}

$k_1(1)$ = 1.0 mU min^{-1} (mg/100 ml) glucose concentration
= 18000.0 mU min^{-1} mol^{-1}

$k_1(2)$ = 2.0 mU min^{-1} (mg/100 ml min^{-1})
= 36000.0 mU min^{-1} (mol min^{-1})

f_{p_1} - Using the experimental glucose and insulin following intravenous testing to yield values for insulin level glucose elevation and rate of change together with the estimates for k_{10}, k_1 and k_{12} substitution in the insulin controller equation enabled values of the insulin less function to be obtained as listed in Table V.

$$f_{12}(X_1) = \frac{12.5\ X_1}{22.7 + 0.3\ X_1}$$

$$f_{24}(X_2) = \frac{1.25\ X_2}{0.10722 + 1848.3\ X_2}$$

$$f_{42}(X_2) = \frac{0.03 - 31.2\ X_2}{32.0 - 1000.0\ X_2} + \frac{11.1}{4000.0\ X_2 + 1.0}$$

$K_{16} = 0.000139$ mol m^{-1}

$K_{C5} = 0.23$ mol m^{-1}

$k_{21} = 7.5\ m^{-1}$

$k_{32} = 1177.0\ m^{-1}$

$k_{23} = 23.1\ m^{-1}$

$k_{53} = 200\ m^{-1}$

$k_{35} = 2950\ m^{-1}$

$k_{5a} = 3.39\ m^{-1}$

$K_{Io} = 4.0$ mU min^{-1}

$K_{Eo} = 0.9$ mg min^{-1}

$k_{I(1)} = 18000$ mU min^{-1} mol^{-1}

$k_{I(2)} = 36000$ mU mol^{-1}

$k_{J(1)} = 3600\ \mu g$ min^{-1} mol^{-1}

$k_{J(2)} = 3600\ \mu g$ mol^{-1}

$k_{J(3)} = 0.625\ m^{-1}$

$k_{E(1)} = 3.6$ mg min^{-1} mol^{-1}

$k_{E(2)} = 1.8$ mg mol^{-1}

$k_{E(3)} = 2.81\ m^{-1}$

$K_{Jo} = 0.2$ ug min^{-1}

$X'_1 = 0.0045$ mol

$X'_6 = 40.0$ mU

$X'_7 = 0.48$ ug

$X'_8 = 0.32$ mg

Table IV Details of the non-linear functions, values for rate constants, basal secretion rates and physiological threshold values.

I_p1 (m U)	$f_{p_I}1$ (I_p) (m U/min)
0.0	4.0
86.4	6.3
112.0	7.3
128.0	15.7
150.4	39.8
176.0	61.3
217.6	93.7
230.6	115.0
272.0	174.0
291.0	227.2

Table V

k_{J0} = 2.0 l/min x 100.0 ng/l = 0.2 ug/min

k_{J1} = 1000.0 ug/min^{-1} mol^{-1}

k_{J2} = 1000.0 ug/min^{-1} (mol min^{-1})

k_{J3} = 0.625 min^{-1}

k_{E0} = 0.9 mg min^{-1}

k_{E1} = 3.6 mg min^{-1} mol^{-1}

k_{E2} = 1.8 mg min^{-1} (mol min^{-1})

$k_{PE}1$ = 2.81 min^{-1}

Threshold values:

Insulin = 32.0 mU

Glucagon = 0.48 µg

Adrenalin = 0.32 mg

Initial conditions:

The normal ranges for the steady state are as follows :

Glucose 4.0 - 5.5 mmol/l

Glucose-6-phosphate 100.0 - 220.0 nmol/g liver

Triose phosphate	2.5 - 5.0 nmol/g liver
Glycogen	250 µmol/ml liver
Pyruvate	30.0 - 200.0 nmol/g liver
Insulin	5.0 - 15.0 µU/ml
Glucagon	75.0 - 150.0 µg/ml
Adrenalin	0.01 - 0.1 µg/ml

Assuming a liver mass of 1.5 kg and a plasma volume of 3.21 values within these ranges were chosen, compatible with a steady state solution of the model viz.

Glucose	= 0.005 mol
Glucose-6-phosphate	= 0.0003 mol
Triose phosphate	= 0.000006 mol
Glycogen	= 0.25 mol
Pyruvate	= 0.00122 mol
Insulin	= 34.0 mU
Glucagon	= 0.32 µg
Adrenalin	= 0.16 mg

Hormone basal secretion rates were calculated from the relationship:

metabolic clearance rate mean plasma concentration = basal secretion rate.

The test stimuli were calculated as follows:

Intravenous glucose injection

0.5g glucose/kg body weight is administered over a period of 2 minutes. Assuming a mass of 70 kg body weight this represents the administration of 35/180 moles of glucose over this period.

$\therefore$ The dose = 0.0304 mol min^{-1}

Primed glucose infusion

A priming impulse of 15 cm^3 of a 0.5 g/ml glucose solution is applied over the first 3 minutes of the infusion. Thereafter the glucose solution is infused at the rate of 30 ml hr^{-1}

$\therefore$ Dose rate = 0.00435 mol min^{-1} (0–3 min)

= 0.000434 mol min^{-1} (from then on)

Intravenous insulin injection

0.1 U insulin/kg body weight is administered over a period of 1 minute.

$\therefore$ Dose rate = 7000.0 mU min^{-1}

At this stage it was realised after preliminary computer runs that the model was going to provide problems of computer simulation arising from the stiff nature of the set of differential equations. It was observed in these preliminary runs that contrary to expectation those phosphate and pyruvate were not dominating substrates. In the simulations their dynamics were transient and disappeared very rapidly before regaining the steady state. This seemed adequate grounds for removal of these parameters from the equation set reducing the stiffness. This reduced model was subsequently used for all the work described, though three other features were included to improve physiological likeness.

i) The model was modified to incorporate a specific switching effect either for glycogen synthesis from glucose-6-phosphate or for the latter metabolism via the glycolytic pathway depending upon whether glycogen storage was saturated or not. This saturation concentration was set at 0.5 mol, a value twice that of the initial condition.

ii) Glucagon control of the glucose-6-phosphate to glycogen sequence was also introduced so that when glucagon is elevated above a threshold value this sequence is inhibited; a situation only resolved when glucagon falls below the critical level. Such a step was necessary to prevent substrate recycling through the sequence:

glucose-6-phosphate → UDPG → glycogen → glucose-6-phosphate.

iii) It was further realised that for realistic simulation of blood glucose dynamics account had to be taken of glucose transport into peripheral tissues. It was assumed that approximately 20% - 25% of the uptake of glucose would be accounted for by peripheral uptake. Membrane transport mechanisms involved are regulated by insulin and it is necessary for a minimal insulin level to be attained for initiation of the process of uptake. However, it is suggested that over a wide range of insulin values upwards from this level transport will be controlled by the enzyme dynamics of hexokinase. Insulin then adopts a permissive role.

The rate of production of glucose-6-phosphate is glucose concentration dependent as depicted in Figure 17. The hyperbolic slope of the curve shows the asymptotic approach to maximal production rate of glucose-6-phosphate as glucose concentration tends towards infinity. The dynamics of the reaction are similar for both glucokinase and hexokinase catalysed reactions the maximum velocity being different.

The insulin transport effect is effectively modelled by a multiplier function. That is 'effective' glucose concentration is multiplied by a factor proportional to insulin elevation above the high threshold thus moving the reaction towards its maximal velocity.

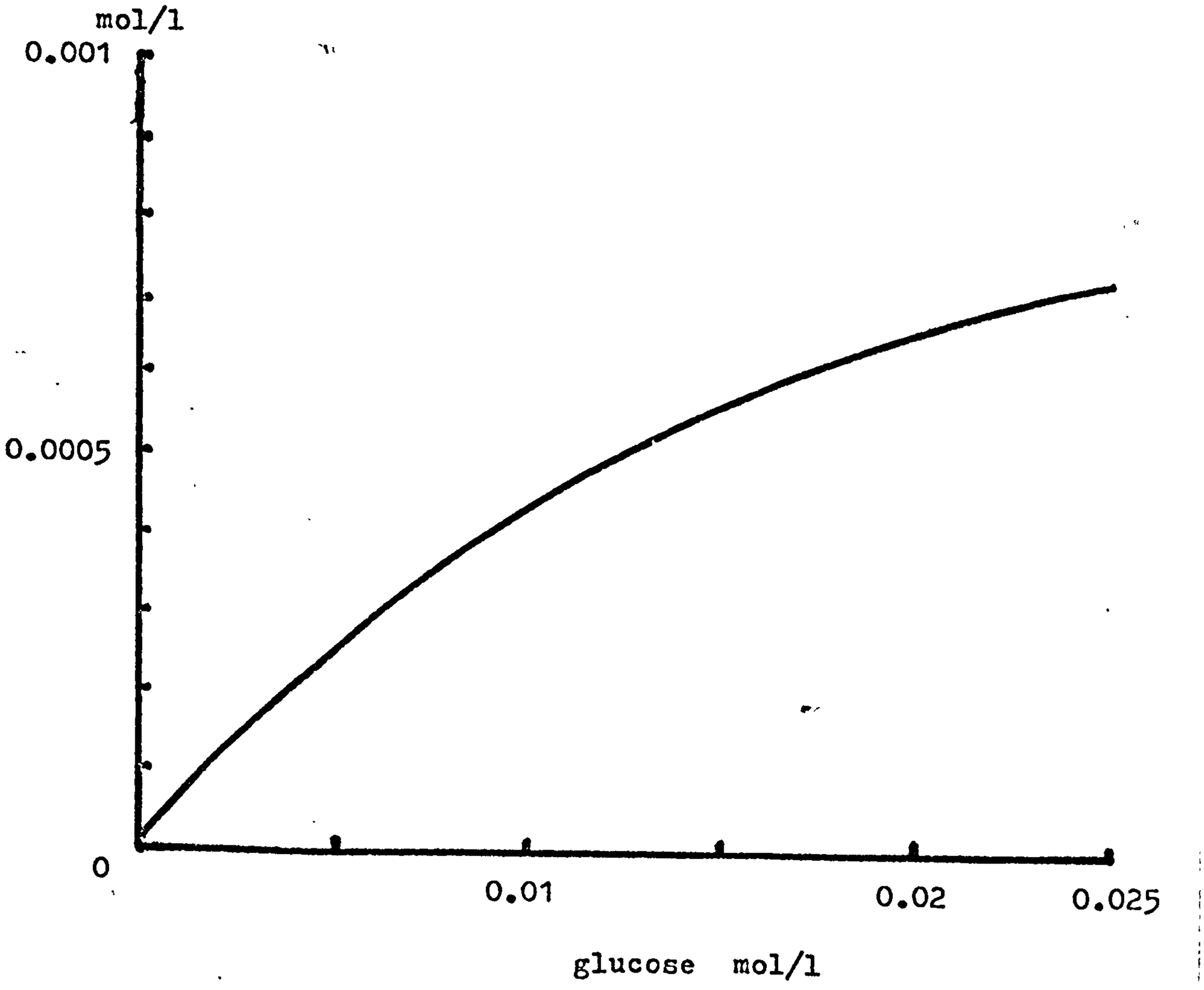


Figure 17

Graph showing the dependence of the rate of production of glucose-6-phosphate upon glucose concentration.

CHAPTER 6

TESTING THE MODEL

The mathematical model was implemented and tested using the CDC 6600 and later the CDC 7600 computer at the University of London Computer Centre. The programs for this purpose were initially written in the digital simulation language MIMIC but were subsequently re-written in FORTRAN because of deletion of MIMIC from the compiler. This step would have been necessary in any case in an attempt to economise on computer time. The program and its sub-routines are presented in facsimiles of the computer print-outs on pages 98 to 101 . The integration sub-routine involved the use of a fourth-order variable step Runge-Kutta technique.

The model was subjected to a series of impulses test, representing intravenour glucose infusion, the intravenous glucose tolerance test, intravenous injection of insulin and injection of glucagon. The model was then progressively modified until acceptable simulations were obtained either, by adding further equations or by choosing different parameter values. A full list of the modifications is given in Table VI.

A section of a typical computer output is shown in a facsimile of the print-out on page 105 .

```
 24/05/73 UNIVERSITY OF MINNESOTA 7600 FORTRAN COMPILER SCOPE V2.0    PSR10    21/06/74  20.49.15
      MNF(P=5000)
 0001003      1*        PROGRAM GNON(INPUT,OUTPUT,TAPE1=INPUT,TAPE2=OUTPUT)
SEQ     1* NOTE - PROGRAM STATEMENT IS NON STANDARD     ***************************************************
                C     GLUCOSE MODEL 5
 0001428      2*        COMMON X(15),DX(15),N,T,DT,DTMIN,P(10),FCO,FDC,F1IP,F2IP,FI,PL,
                      1 F1EP,F2EP
 0001429      3*        READ(1,100) N
                C     N = NO. OF VARIABLES IN MODEL
 0001478      4*    100 FORMAT(I2)
                C     X(1)=GP,X(2)=MC,X(3)=LD,X(4)=IP,X(5)=JP,X(6)=EP
 0001479      5*        READ(1,101) DT1,TMAX,TMIN
                C     DT1=INIT. INTEGR. STEP, TMAX = FINAL TIME, TMIN = START TIME
 0001568      6*    101 FORMAT(3F10.6)
 0001569      7*        DTMIN=.000003
 0001563      8*        CC8=.00003
 0001603      9*        T=TMIN
 0001618     10*        TWRIT=0.
 0001623     11*        DT=DT1
 0001648     12*        READ(1,102) (X(I),I=1,N)
 0001778     13*    102 FORMAT(6F10.6)
 0001778     14*        READ(2,111) (P(I),I=1,10)
 0002108     15*    111 FORMAT(7F10.3/3F10.3)
                C     P(1)=GAIN RELATING GP ELEVATION TO IP
                C     P(2)=GAIN RELATING GP RATE TO IP
                C     P(3)=GAIN RELATING GP ELEVATION TO JP
                C     P(4)=GAIN RELATING GP RATE TO JP
                C     P(5)=IP THRESHOLD
                C     P(6)=JP THRESHOLD
                C     P(7)=JP LOSS RATE CONSTANT
                C     P(8)=GAIN RELATING GP DEPRESSION TO EP
                C     P(9)=GAIN RELATING GP RATE TO EP
                C     P(10)=EP THRESHOLD
 0002108     16*        WRITE(2,112) (P(I),I=1,10)
 0002218     17*    112 FORMAT(1H ,7HP(1) = ,F10.3,4X,7HP(2) = ,F10.3,4X,7HP(3) = ,F10.3//
                      1 7HP(4) = ,F10.3,4X,7HP(5) = ,F10.3,4X,7HP(6) = ,F10.3,4X,
                      2 7HP(7) = ,F10.3//7HP(8) = ,F10.3,4X,7HP(9) = ,F10.3,4X,
                      3 7HP(10) = ,F10.3)
 0002218     18*        WRITE(2,103) T,X(1),X(2),X(3),X(4),X(5),X(6)
 0002340     19*    103 FORMAT(1H0,4HT = ,1PE13.6,3X,5H GP  ,1PE11.3,6H  MC  ,1PE11.3,
                      1 6H  LD  ,1PE11.3,6H  IP  ,1PE11.3,6H  JP  ,1PE11.3,6H  EP  ,
                      2 1PE11.3/)
 0002343     20*        GO TO 106
 0002348     21*    107 TT=T-TWRIT
 0002368     22*        IF(TT.GE.0.1.AND.T.LE.10.) GO TO 104
 0002423     23*        IF(TT.GE.1.0.AND.T.GT.10.) GO TO 104
 0002468     24*        GO TO 108
 0002469     25*    104 WRITE(2,105) T,X(1),X(2),X(3),X(4),X(5),X(6),DX(1),DX(2),DX(3),
                      1 DX(4),DX(5),DX(6),FCO,FDC,F1IP,F2IP,FI,PL
 0002763     26*    105 FORMAT(1H0,4HT = ,1PE13.6,3X,5H GP  ,1PE11.3,6H  MC  ,1PE11.3,
                      1 6H  LD  ,1PE11.3,6H  IP  ,1PE11.3,6H  JP  ,1PE11.3,6H  EP  ,
                      2 1PE11.3/21X,6HDGP  ,1PE11.3,6H DMC  ,1PE11.3,6H DLD  ,1PE11.3,
                      3 6H DIP  ,1PE11.3,6H DJP  ,1PE11.3,6H DEP  ,1PE11.3/21X,6H FCO  ,
                      4 1PE11.3,6H FDC  ,1PE11.3,6HF1IP  ,1PE11.3,6HF2IP  ,1PE11.3,
                      5 6H  FI  ,1PE11.3,6H  PL  ,1PE11.3/)
 0002763     27*        TWRIT=T
 0002763     28*    108 IF(T.LT.CC8) DT=DT1
 0003043     29*        IF(T.GE.CC8) DT=.01
 0003078     30*    106 CALL INTEGR
 0003103     31*        IF(T.LT.TMAX) GO TO 107
 0003128     32*        STOP
```

Figure 18

The Fortran program used for simulation of hepatic glucose model incorporating the master segment of the Runge-Kutta sub-routine and the model sub-routines.

```
0013758      81*          SUBROUTINE MODEL
0013758      82*          REAL IIV,JIV
0013758      83*          COMMON X(15),DX(15),N,T,DT,DTMIN,P(10),FCO,FDC,F1IP,F2IP,FI,PL,
                         1 F1EP,F2EP
                    C    NO INPUT
0013758      84*          GIV=0.
0013778      85*          IIV=0.
0013778      86*          JIV=0.
0014008      87*          EIV=0.
0014018      88*          FDC=7.7*X(1)/(22.7+1150.*X(1))
0014058      89*          PL=0.
0014068      90*          IF(X(4).GT.45.0.AND.X(4).LE.700.) PL=2.5*X(1)/(22.7+1150.*X(1))
0014178      91*          IF(X(4).GT.700.) XP=X(1)+X(1)*(X(4)-700.)
0014238      92*          IF(X(4).GT.700.) PL=2.5*XP/(22.7+1150.*XP)
0014318      93*          DX(1)=GIV-.000139+7.5*X(2)-FDC-PL
0014388      94*          FCO=1.25*X(2)/(.10722+1848.3*X(2))
0014428      95*          IF(X(4).LT.P(5).OR.X(3).GT.0.5) FCO=0.
0014508      96*          TFDC=(.33-31.2*X(2))/(32.-1000.*X(2)+11.1/(4000.*X(2)+1.))
0014618      97*          FDC=TFDC
0014628      98*          IF(X(5).LT.P(6).AND.X(6).LT.P(10)) FDC=0.
0014678      99*          DX(2)=.00706-7.5*X(2)+FDC-23.1*X(2)-FCO+FDC
0014758     100*          DX(3)=FCO-FDC
0014768     101*          F1IP=0.
0014778     102*          IF(X(1).GT.0.0045) F1IP=X(1)-.0045
0015038     103*          F2IP=0.
0015038     104*          IF(DX(1).GT.0.) F2IP=DX(1)
0015068     105*          F1EP=0.
0015068     106*          IF(X(1).LT.0.0045) F1EP=.0045-X(1)
0015128     107*          F2EP=0.
0015128     108*          IF(DX(1).LT.0.) F2EP=-DX(1)
0015158     109*          XSUB=X(4)
0015158     110*          CALL FINS(XSUB,FI)
0015218     111*          X(4)=XSUB
0015218     112*          DX(4)=4.*IIV+P(1)*F1IP+P(2)*F2IP-FI
0015318     113*          DX(5)=.2*JIV+P(3)*F1IP+P(4)*F2IP-P(7)*X(5)
0015408     114*          DX(6)=0.9*EIV+P(8)*F1EP+P(9)*F2EP-5.62*X(6)
0015508     115*          RETURN
0015528     116*          END
SEQ   116* COMMENT - 1 WORDS USED IN SETUP OF SUBPROGRAM   ************************************************

NUMBER AND NAME CROSS REFERENCE MAP,  /N=REPEATED N TIMES
 NAME TYPE MAIN USE ADDRESS  BLOCK U=USED, S=STORED, D=DECLARED, I=DO INDEX, A=ASSIGN, P=DO PARAMETER, *=NULL
DX      R ARRAY       0000178 //        115S 114S 113S 108U/2 104U/2 101S 100S 94S 83D
EIV     R VARIABLE    0016158           114U 88S
FCO     R VARIABLE    0000E48 //        100U 99U 95S/2 83D
FDC     R VARIABLE    0000558 //        100U 99U 98S/2 83D
FI      R VARIABLE    0000608 //        112U 110U 83D
FINS    R EXTERNAL                      110U
FPC     R VARIABLE    0016178           99U 93U 89S
F1EP    R VARIABLE    0000628 //        114U 106S/2 83D
F1IP    R VARIABLE    0000568 //        113U 112U 102S/2 83D
F2EP    R VARIABLE    0000638 //        114U 108S/2 83D
F2IP    R VARIABLE    0000578 //        113U 112U 104S/2 83D
GIV     R VARIABLE    0016138           93U 85S
IIV     R VARIABLE    0013758           112U 86S 82D
JIV     R VARIABLE    0013768           113U 87S 82D
P       R ARRAY       0000428 //        114U/2 113U/3 112U/2 98U/2 95U 83D
PL      R VARIABLE    0000518 //        93U 92S 90S/2 83D
TFDC    R VARIABLE    0016148           97S 97U
X       R ARRAY       0000008 //        114U 113U 112S 109U 106U/2 102U/2 99U/2 98U/2 96U/3 95U/2 94U/2 93U 32U 91U/4 90U/4
```

Figure 18 [cont'd]

Sub-routine MODEL

```
0004648      34*        SUBROUTINE INTEGR
0004648      35*        REAL K1,K2,K3,K4
0004648      36*        COMMON X(15),DX(15),N,T,DT,DTMIN,P(10),FCD,FDC,F1IP,F2IP,FI,PL,
                       1 F1EP,F2EP
0004648      37*        DIMENSION K1(50),K2(50),K3(50),K4(50),XS(50),XZ(50),OLD(50)
0004648      38*        ERR=0.001
0012228      39*        DO 60 I=1,N
0012258      40*     60 XS(I)=X(I)
0012278      41*        TO=T
0012308      42*        H=DT
0012318      43*        NO=1
0012338      44*      5 DO 2 J=1,NO
0012368      45*        CALL MODEL
0012408      46*        DO 10 I=1,N
0012428      47*     10 XZ(I)=X(I)
0012448      48*        T=T+0.5*H
0012478      49*        DO 20 I=1,N
0012503      50*        K1(I)=H*DX(I)
0012513      51*     20 X(I)=XZ(I)+0.5*K1(I)
0012553      52*        CALL MODEL
0012578      53*        DO 30 I=1,N
0012613      54*        K2(I)=H*DX(I)
0012628      55*     30 X(I)=XZ(I)+0.5*K2(I)
0012653      56*        CALL MODEL
0012708      57*        DO 40 I=1,N
0012728      58*        K3(I)=H*DX(I)
0012733      59*     40 X(I)=XZ(I)+K3(I)
0012769      60*        T=T+0.5*H
0013008      61*        CALL MODEL
0013028      62*        DO 2 I=1,N
0013048      63*        K4(I)=H*DX(I)
0013058      64*      2 X(I)=XZ(I)+(K1(I)+2.0*(K2(I)+K3(I))+K4(I))/6.
0013208      65*        IF(NO.EQ.1) GO TO 3
0013218      66*        DO 50 I=1,N
0013238      67*        IF(ABS(OLD(I)-X(I)).GE.ABS(ERR*X(I))) GO TO 3
SEQ    67* CAUTION - VARIABLE OR ARRAY - OLD NOT DEFINED AT THIS POINT  ******************************
0013318      68*     50 CONTINUE
0013343      69*        GO TO 4
0013358      70*      3 H=H*0.5
0013367      71*        IF(H.LT.DTMIN) GO TO 6
0013403      72*        DO 70 I=1,N
0013423      73*        OLD(I)=X(I)
0013428      74*     70 X(I)=XS(I)
0013458      75*        T=TO
0013468      76*        NO=NO*2
0013478      77*        GO TO 5
0013503      78*      6 CONTINUE
SEQ    78* COMMENT - CONTINUE STATEMENT IS NOT DO TERMINATOR  ************************************
0013513      79*      4 RETURN
0013528      80*        END
SEQ    80* COMMENT - 1 WORDS USED IN SETUP OF SUBPROGRAM  ****************************************************

NUMBER AND NAME CROSS REFERENCE MAP,  /N=REPEATED N TIMES
NUMBER TYPE ADDRESS  SN=STATEMENT NUMBER, FN=FORMAT NUMBER, J=JUMP, R=READ, W=WRITE, L=LOCATED, D=DO END, A=ASSIGN, T=TRACE
     2 SN    0013056 64L 62D 44D
     3 SN    0013353 70L 67J 65J
     4 SN    0013518 79L 63J
     5 SN    0012343 77J 44L
     6 SN    0013518 79L 71J
    10 SN    0012428 47L 46D
```

Figure 18 [cont'd]

Sub-routine INTEGR

```
 0016213      117*        SUBROUTINE FINS(C,SR1)
 0016218      118*        DIMENSION XI(10),FI(10)
 0016218      119*        DATA(XI(I),FI(I),I=1,10)/0.0,4.0,86.4,6.7,112.0,7.3,129.0,15.7,
                         1 150.4,33.5,176.0,61.3,217.6,93.7,230.6,115.0,272.0,174.0,
                         2 291.0,227.2/
 0016213      120*        N=10
SEQ   119* NOTE - NON STANDARD DATA STATEMENT    ********************************************************************
 0016463      121*        IF(C.GE.XI(1)) GO TO 2
 0016513      122*        C=XI(1)
 0016513      123*      2 J=2
 0016523      124*      3 IF(C-XI(J)) 6,5,4
 0016563      125*      4 J=J+1
 0016573      126*        IF(J.LT.N) GO TO 3
 0016613      127*        M=N-1
 0016613      128*        SR1=FI(M)+(FI(N)-FI(M))/(XI(N)-XI(M))*(C-XI(M))
 0016713      129*        GO TO 7
 0016723      130*      5 SR1=FI(J)
 0016743      131*        GO TO 7
 0016753      132*      6 L=J-1
 0016773      133*        SR1=FI(L)+(FI(J)-FI(L))/(XI(J)-XI(L))*(C-XI(L))
 0017063      134*      7 RETURN
 0017113      135*        END
SEQ   135* COMMENT - ARGUMENT - C SHOULD BE SET TO LOCAL VARIABLE FOR FASTER SUBPROGRAM SETUP      **********
SEQ   135* COMMENT - 14 WORDS USED IN SETUP OF SUBPROGRAM  ***************************************************

NUMBER AND NAME CROSS REFERENCE MAP,  /N=REPEATED N TIMES
NUMBER TYPE ADDRESS  SN=STATEMENT NUMBER, FN=FORMAT NUMBER, J=JUMP, R=READ, W=WRITE, L=LOCATED, D=DO END, A=ASSIGN, T=TRACE
    2 SN    0016528 123L 121J
    3 SN    0016533 126J 124L
    4 SN    0016560 125L 124J
    5 SN    0016738 130L 124J
    6 SN    0016760 132L 124J
    7 SN    0017108 134L 131J 129J
 NAME TYPE MAIN USE ADDRESS  BLOCK U=USED, S=STORED, D=DECLARED, I=DO INDEX, A=ASSIGN, P=DO PARAMETER, *=NULL
C       R PARAMETER   0000018          133U 129U 124U 123S 121U 117D
FI      R ARRAY       0016218          133U/3 130U 128U/3 119S 118D
I       I VARIABLE    0016338          119U/3 119I
J       I VARIABLE    0017378          133U/4 132U 130U/2 126S 126U 125U 124U/2 124S
L       I VARIABLE    0017408          133U/4 133S 133U/2
M       I VARIABLE    0017358          128U/4 128S 128U/2
N       I VARIABLE    0017368          128U/4 127U 126U 121S
SR1     R PARAMETER   0000028          134S 131S 129S 117D
XI      R ARRAY       0016348          133U/3 129U/3 124U 122U 121U 119S 118D
BLOCK NAMES AND LENGTHS
        //          0
VARIABLE AND ARRAY NAMES SORTED BY OCTAL ADDRESS, TRAILING R=ABSOLUTE, C=RELATIVE TO BLANK COMMON
  0016218 FI          0016338 I           0016348 XI          0017358 M           0017368 N           0017378 J
  0017408 L
```

Figure 18 [cont'd]

Sub-routine FINS

Run 1	FPC = 12.*	Ip (periph) threshold = 65
	P(3) = P(4) = 1000	
	P(5) = 32	Jp(0) = 0.4
	P(6) = 32	
Run 2	FPC = 12.*	Ip (periph) threshold = 45
	P(3) = P(4) = 1000	
	P(5) = 32	Jp(0) = 0.4
	P(6) = 32	
Run 3	FPC = 10.*	Ip (periph) threshold = 45
	P(3) = P(4) = 1000	
	P(5) = 32	Jp(0) = 0.4
	P(6) = 32	
Run 4	FPC = 10.*	Ip (periph) threshold = 45
	P(3) = P(4) = 300	
	P(5) = 32	Jp(0) = 0.4
	P(6) = 48	
Run 5	FPC = 10.*	Ip (periph) threshold = 45
	P(3) = P(4) = 100	
	P(5) = 32	Jp(0) = .32
	P(6) = 48	
Run 6	FPC = 8.*	Ip (periph) threshold = 45
	P(3) = P(4) = 30	
	P(5) = 32	Jp(0) = .32
	P(6) = 48	
Run 7	FPC = 9.*	Ip (periph) threshold = 45
	P(3) = P(4) = 30	
	P(5) = 32	Jp(0) = .32
	P(6) = 48	

Run 8

As Run 7 except : if 45 Ip 700 , $PL = \frac{1.5\ Gp}{22.7 + 1150\ Gp}$

if Ip 700 $PL = \frac{1.5 * [(Ip-700)/_{300}+1]_{+\ Gp}}{22.7+1150 * \frac{Ip-700}{300} + 1 \quad Gp}$

TABLE VI Progressive modifications to the original mathematical model.

[cont'd]

Run 9

As run 8 except : if Ip 700 $PL = \frac{1.5 * [(Ip-700)/150 + 1] * Gp}{22.7 + 1150 * [\frac{Ip-700}{150} + 1] Gp}$

Run 10

As run 9 except if Ip 700 $PL = \frac{1.5 * [\frac{(Ip-700)}{50} + 1] * Gp}{22.7 + 1150 * [\frac{Ip-700}{50} + 1] Gp}$

Run 11

As run 10 except if Ip 700 $PL = \frac{1.5 * [\frac{Ip-700}{10} + 1] * Gp}{22.7 + 1150 * [\frac{Ip-700}{10} + 1] Gp}$

and IIV = 7000 if 10 t 11 min (i.e. to allow 'false' transient to decay and 'true' steady state to be reached.

Run 12

As run 11 except if Ip 700 $PL = \frac{1.5 * [(Ip-700) + 1] Gp}{22.7 + 1150 [(Ip-700) + 1] Gp}$

Run 13

As run 12 except PL = 3.0 * ....

FPC = 8. * ....

Run 14

As run 12 except PL = 2.5 * ....

FPC = 8. * ....

Run 15

As run 12 except PL = 2.5 * ....

FPC = 7.8 * ...

Run 16 Addition of adrenalin equ.

Glucagon test applied

16(a) as run 16 except FPC = 7.7*

Run 17

GNON run only for 10 t 20 . FCD = 0

Run 18

If Jp 48.0 FCD = 0

Run 19

GNON for 10 t 30 FCD = 0

JINJ Jp(0) = 44

[cont'd]

Run 20

JINJ 3p(0) = 32 Jp' = .36

Sensitivities of Adrenalin eqn. increased by one order of magnitude

---

Run 21

As run 20 except Jp' = .42

Adrenalin eqn. sensitivities : 180 (level) 90 (rate)

Run 22

As run 21 except Jp' = .39

Run 23 (JINJ only) Jp' = .42 as run 21 except :

if Jp Jp' PL = x(1) / (22.7 + 1150 x(1)) i.e. reduction to 40% normal loss rate

---

Run 24

JINJ as run 23 except :

if Jp Jp' PL = 2.* x(1) / (22.7 + 1150 x(1))

Run 25

JINJ only as run 23 except :

if Jp Jp' PL = 1.5 * x(1) / (22.7 + 1150 * x(1))

Run 26

JINJ only as run 23 except :

if Jp Jp' PL = 1.25 * x(1) / (22.7 + 1150 * x(1))

Run 27

JINJ as run 23 except :

if Jp Jp' PL = 1.4 * x(1) / (22.7 + 1150 * x(1))

Run 28

JINJ only as run 23 except :

PL = 1.45 * x(1) / (22.7 + 1150 * x(1))

Run 29

JINJ only as run 23 except :

PL = 1.42 * .....

```
 CTIME =      119MS   PSR 10   CN =   17058 (041242B)
 P(1) =   18000.000   P(2) =  36000.000   P(3) =     30.000
 P(4) =     30.000   P(5) =     32.000   P(6) =      .390   P(7) =      .625
 P(8) =    180.000   P(9) =     90.000   P(10) =     .320

 T =              0    GP   5.000E-03  MC   3.000E-04  LD  2.500E-01  IP  3.400E+01  JP  3.200E-01  EP  1.600E-01

 T =  1.000310E-01    GP   5.053E-03  MC   2.591E-04  LD  2.501E-01  IP  3.676E+01  JP  3.231E-01  EP  1.601E-01
                      DGP  4.308E-04 DMC  -5.705E-05 DLD  5.526E-04 DIP  2.480E+01 DJP  2.786E-02 DEP  4.560E-04
                       FCD  5.526E-04 FDC        0F1IP  5.529E-04F2IP  4.398E-04  FI  4.979E+00  PL           0

 T =  2.000310E-01    GP   5.096E-03  MC   2.576E-04  LD  2.501E-01  IP  3.923E+01  JP  3.258E-01  EP  1.601E-01
                      DGP  4.190E-04 DMC   2.189E-07 DLD  5.520E-04 DIP  2.476E+01 DJP  2.681E-02 DEP  2.599E-04
                       FCD  5.520E-04 FDC        0F1IP  5.955E-04F2IP  4.190E-04  FI  5.044E+00  PL           0

 T =  3.100310E-01    GP   5.141E-03  MC   2.578E-04  LD  2.502E-01  IP  4.198E+01  JP  3.287E-01  EP  1.601E-01
                      DGP  4.110E-04 DMC   2.760E-06 DLD  5.521E-04 DIP  2.522E+01 DJP  2.612E-02 DEP  1.401E-04
                       FCD  5.521E-04 FDC        0F1IP  6.412E-04F2IP  4.110E-04  FI  5.117E+00  PL           0

 T =  4.200310E-01    GP   5.186E-03  MC   2.581E-04  LD  2.502E-01  IP  4.478E+01  JP  3.315E-01  EP  1.601E-01
                      DGP  4.037E-04 DMC   2.786E-06 DLD  5.522E-04 DIP  2.569E+01 DJP  2.547E-02 DEP  7.549E-05
                       FCD  5.522E-04 FDC        0F1IP  6.860E-04F2IP  4.037E-04  FI  5.192E+00  PL           0

 T =  5.300310E-01    GP   5.180E-03  MC   2.582E-04  LD  2.503E-01  IP  4.614E+01  JP  3.331E-01  EP  1.605E-01
                      DGP -4.761E-05 DMC  -1.928E-07 DLD  5.522E-04 DIP  1.110E+01 DJP  1.237E-02 DEP  2.446E-03
                       FCD  5.522E-04 FDC        0F1IP  6.848E-04F2IP           0  FI  5.228E+00  PL  4.522E-04

 T =  6.300310E-01    GP   5.180E-03  MC   2.582E-04  LD  2.503E-01  IP  4.724E+01  JP  3.343E-01  EP  1.607E-01
                      DGP -4.649E-05 DMC  -3.155E-07 DLD  5.522E-04 DIP  1.098E+01 DJP  1.149E-02 DEP  1.318E-03
                       FCD  5.522E-04 FDC        0F1IP  6.801E-04F2IP           0  FI  5.258E+00  PL  4.519E-04

 T =  7.300310E-01    GP   5.175E-03  MC   2.581E-04  LD  2.504E-01  IP  4.833E+01  JP  3.354E-01  EP  1.607E-01
                      DGP -4.544E-05 DMC  -3.142E-07 DLD  5.522E-04 DIP  1.087E+01 DJP  1.066E-02 DEP  6.784E-04
                       FCD  5.522E-04 FDC        0F1IP  6.755E-04F2IP           0  FI  5.287E+00  PL  4.516E-04

 T =  8.300310E-01    GP   5.171E-03  MC   2.581E-04  LD  2.505E-01  IP  4.942E+01  JP  3.364E-01  EP  1.608E-01
                      DGP -4.440E-05 DMC  -3.074E-07 DLD  5.522E-04 DIP  1.076E+01 DJP  9.880E-03 DEP  3.150E-04
                       FCD  5.522E-04 FDC        0F1IP  6.710E-04F2IP           0  FI  5.315E+00  PL  4.513E-04

 T =  9.300310E-01    GP   5.167E-03  MC   2.581E-04  LD  2.505E-01  IP  5.049E+01  JP  3.374E-01  EP  1.608E-01
                      DGP -4.339E-05 DMC  -3.005E-07 DLD  5.522E-04 DIP  1.065E+01 DJP  9.154E-03 DEP  1.104E-04
                       FCD  5.522E-04 FDC        0F1IP  6.666E-04F2IP           0  FI  5.344E+00  PL  4.510E-04

 T =  1.030031E+00    GP   5.162E-03  MC   2.580E-04  LD  2.506E-01  IP  5.155E+01  JP  3.382E-01  EP  1.608E-01
                      DGP -4.240E-05 DMC  -2.938E-07 DLD  5.522E-04 DIP  1.055E+01 DJP  8.474E-03 DEP -5.012E-06
                       FCD  5.522E-04 FDC        0F1IP  6.623E-04F2IP           0  FI  5.372E+00  PL  4.507E-04

 T =  1.140031E+00    GP   5.158E-03  MC   2.580E-04  LD  2.506E-01  IP  5.270E+01  JP  3.391E-01  EP  1.608E-01
                      DGP -4.134E-05 DMC  -2.865E-07 DLD  5.522E-04 DIP  1.044E+01 DJP  7.778E-03 DEP -7.385E-05
                       FCD  5.522E-04 FDC        0F1IP  6.577E-04F2IP           0  FI  5.403E+00  PL  4.504E-04
```

Figure 19

Typical print-out of the computer output

CHAPTER 7

RESULTS

On the subsequent pages are depicted in graphical form the results of progressive simulations (Figures 20-57). The conventions adopted are as follows :

Glucose	;	mmol/l	●
Insulin	;	mU/3.2 l	▲
Glucagon	;	µg/3.2 l	■
Adrenaline	;	mg/3.2 l	◆

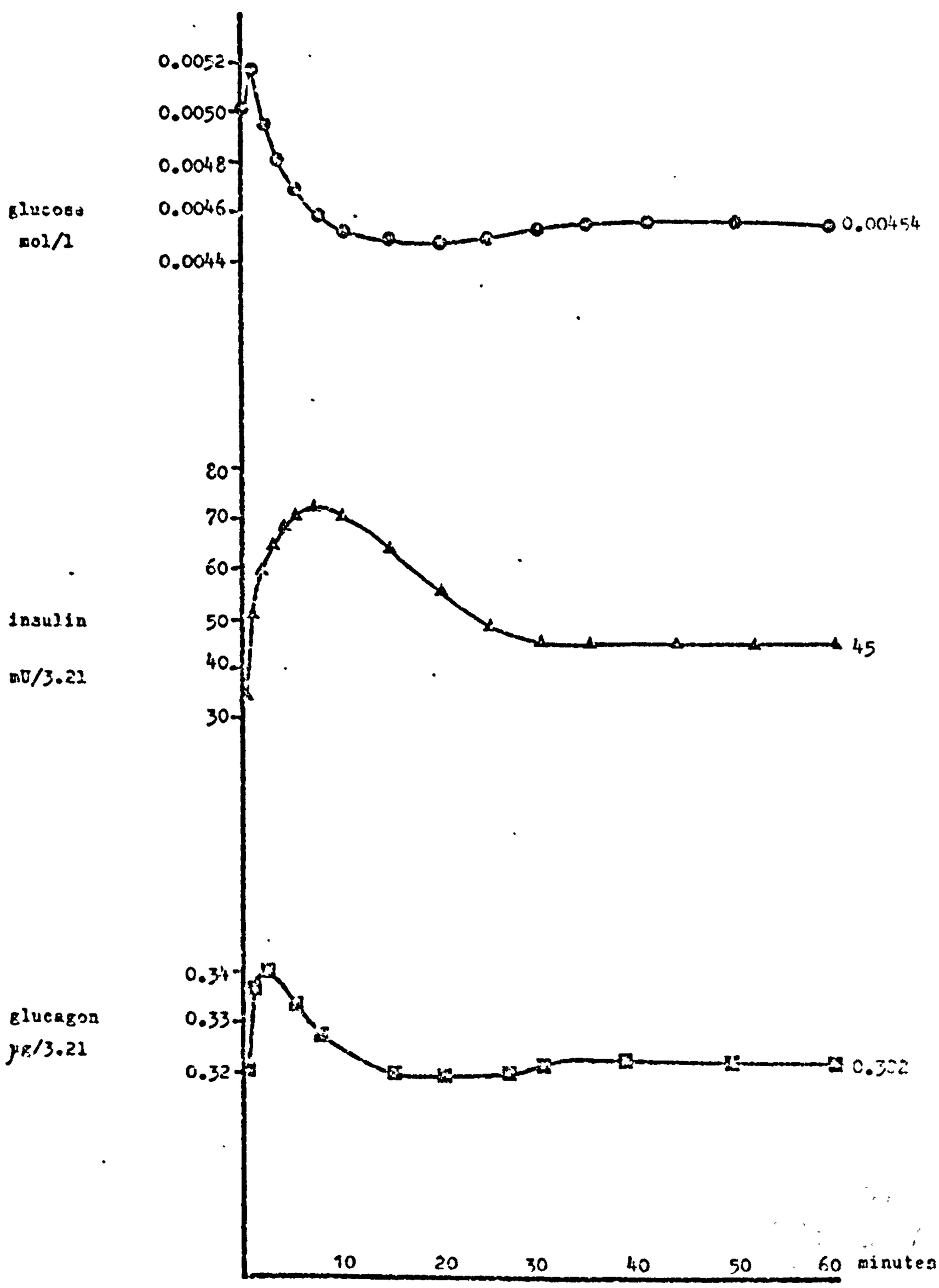


Figure 20 Result of model simulation without any input. Conditions set as in run 17.

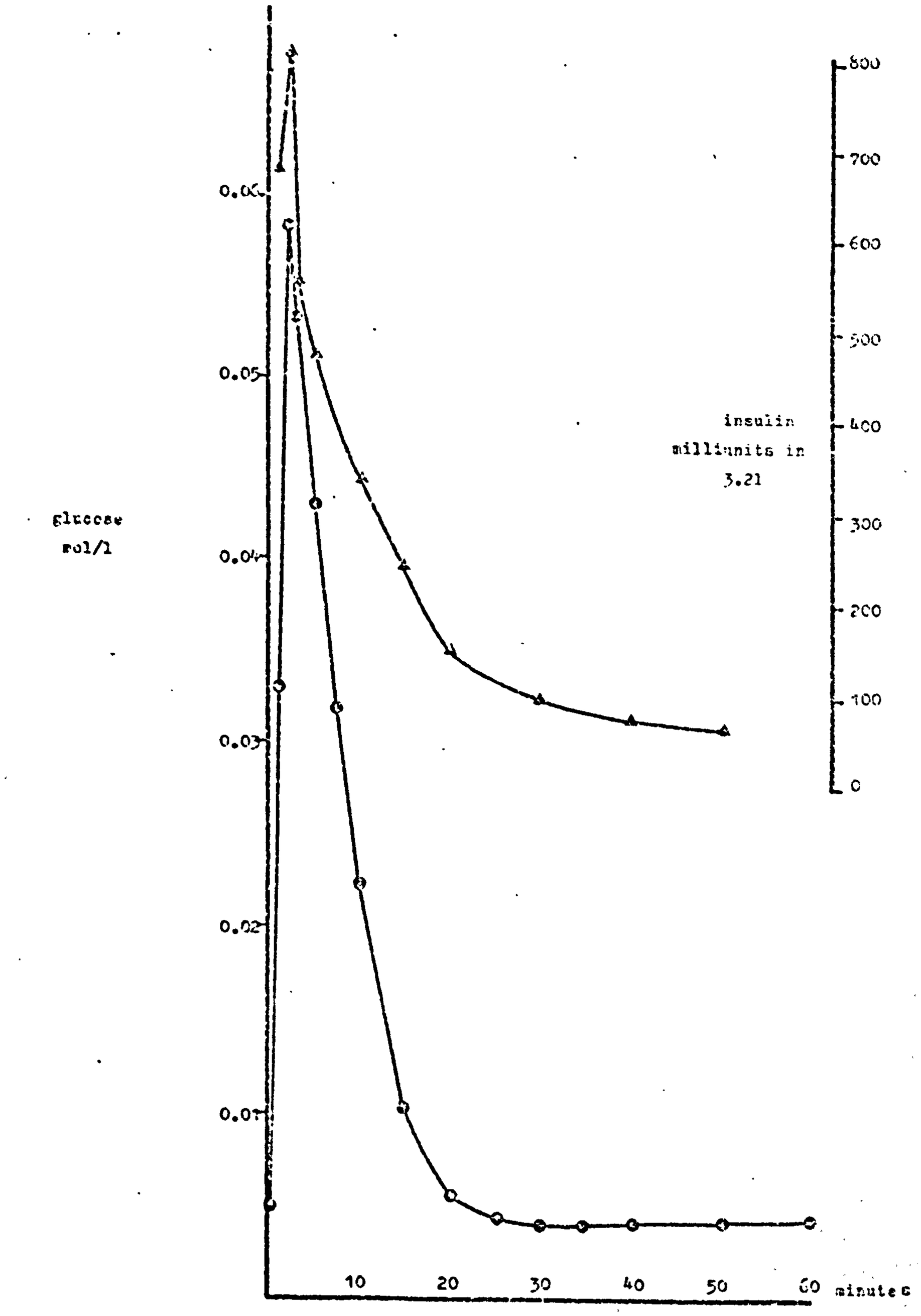


Figure 21 Simulation of intravenous glucose tolerance test. Conditions set as in run 2. Not acceptable.

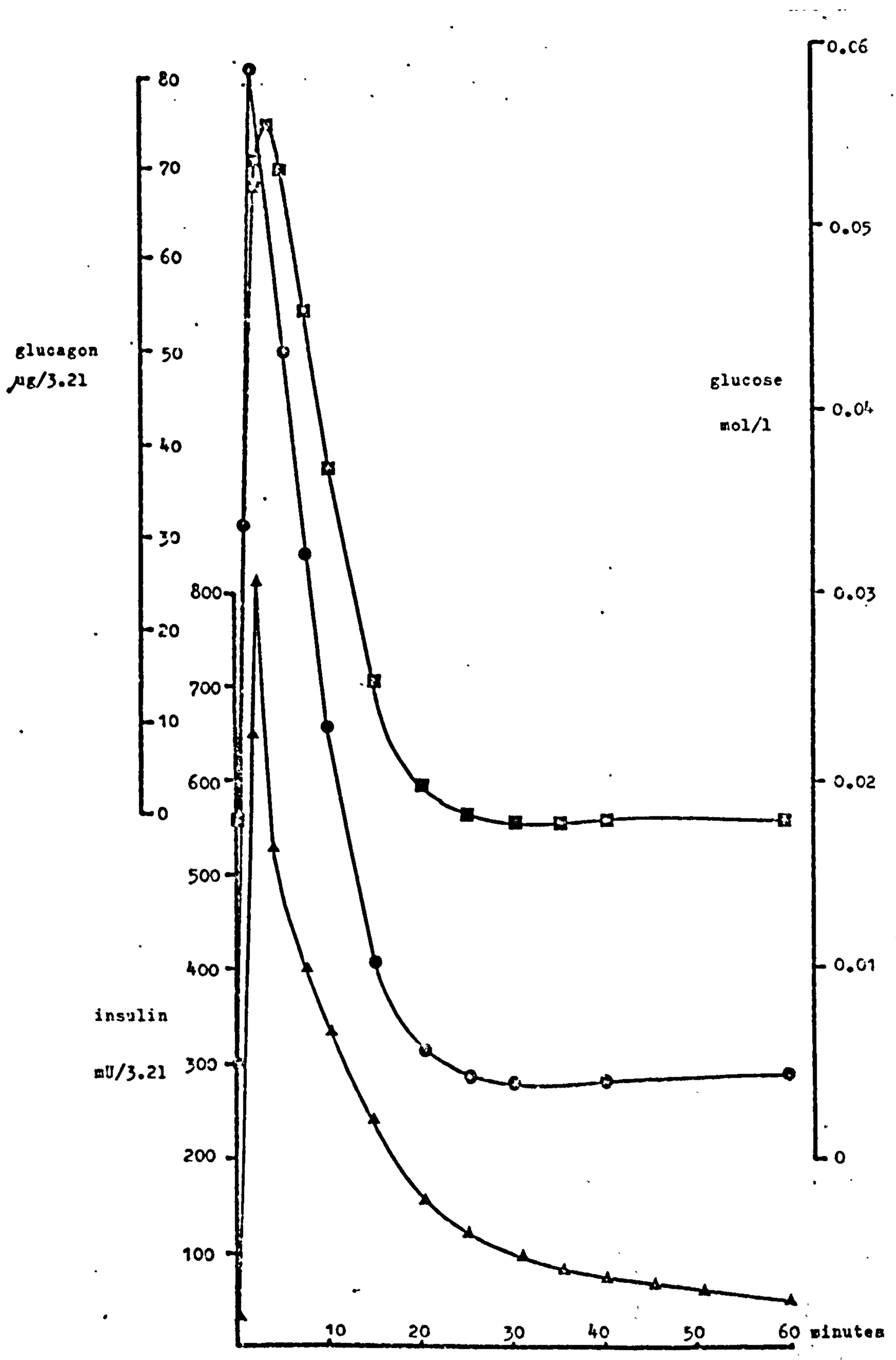


Figure 22 Simulation of intravenous glucose tolerance test. Conditions set as in run 6. Not acceptable.

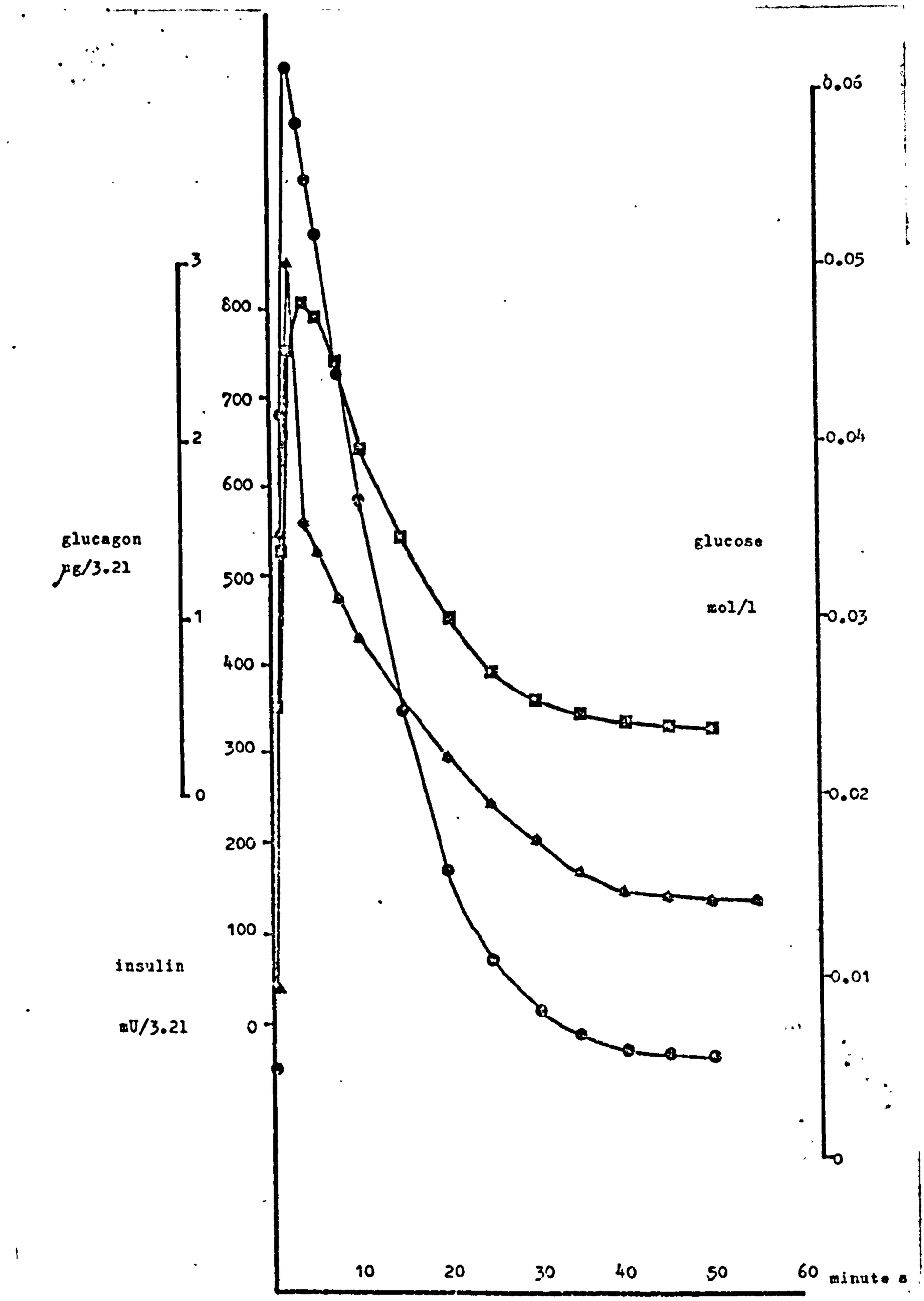


Figure 23 Simulation of intravenous glucose tolerance test. Conditions set as in run 9. Not acceptable.

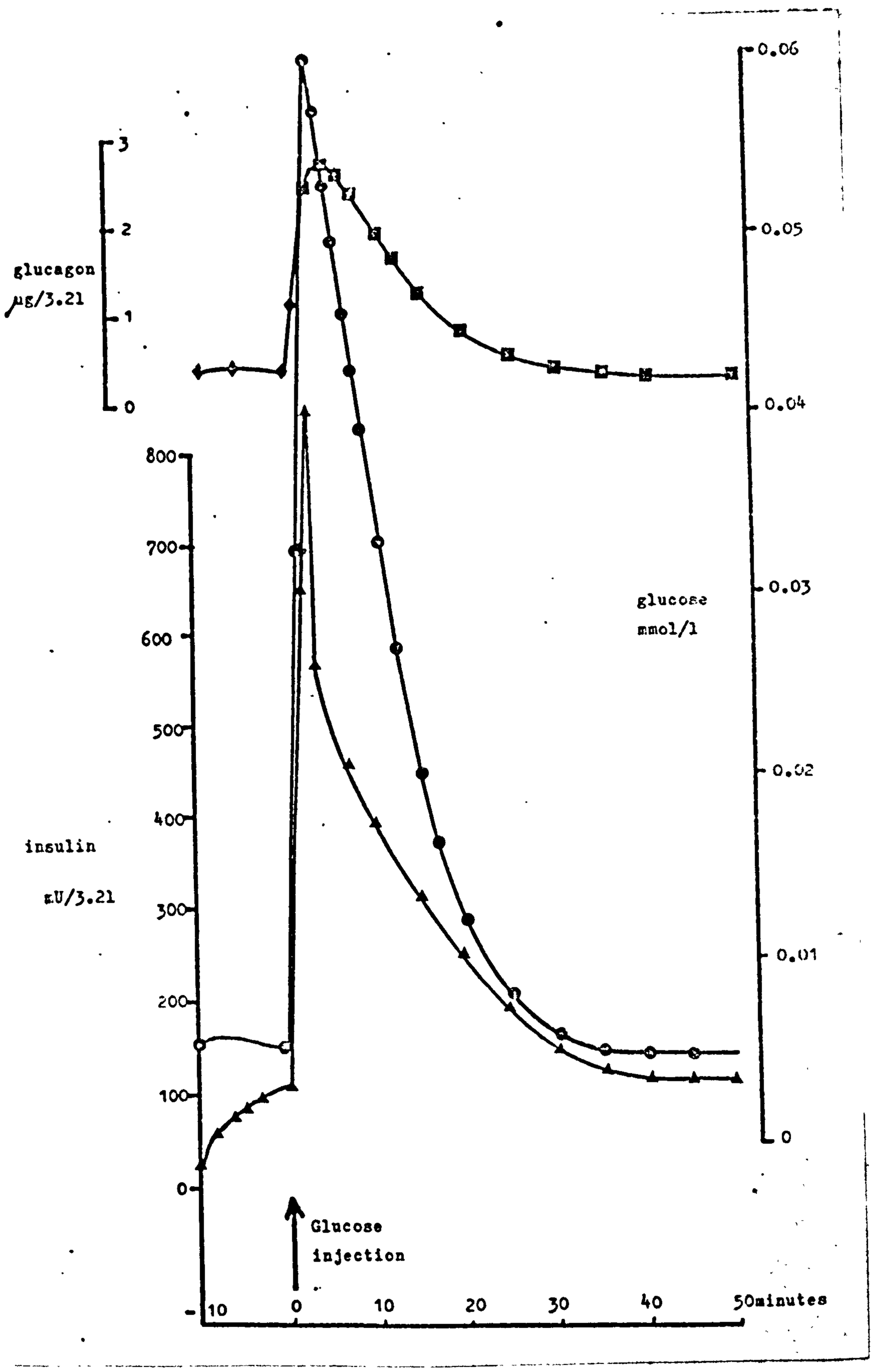


Figure 24. Simulation of intravenous glucose tolerance test. Conditions set as in run 11. Not acceptable.

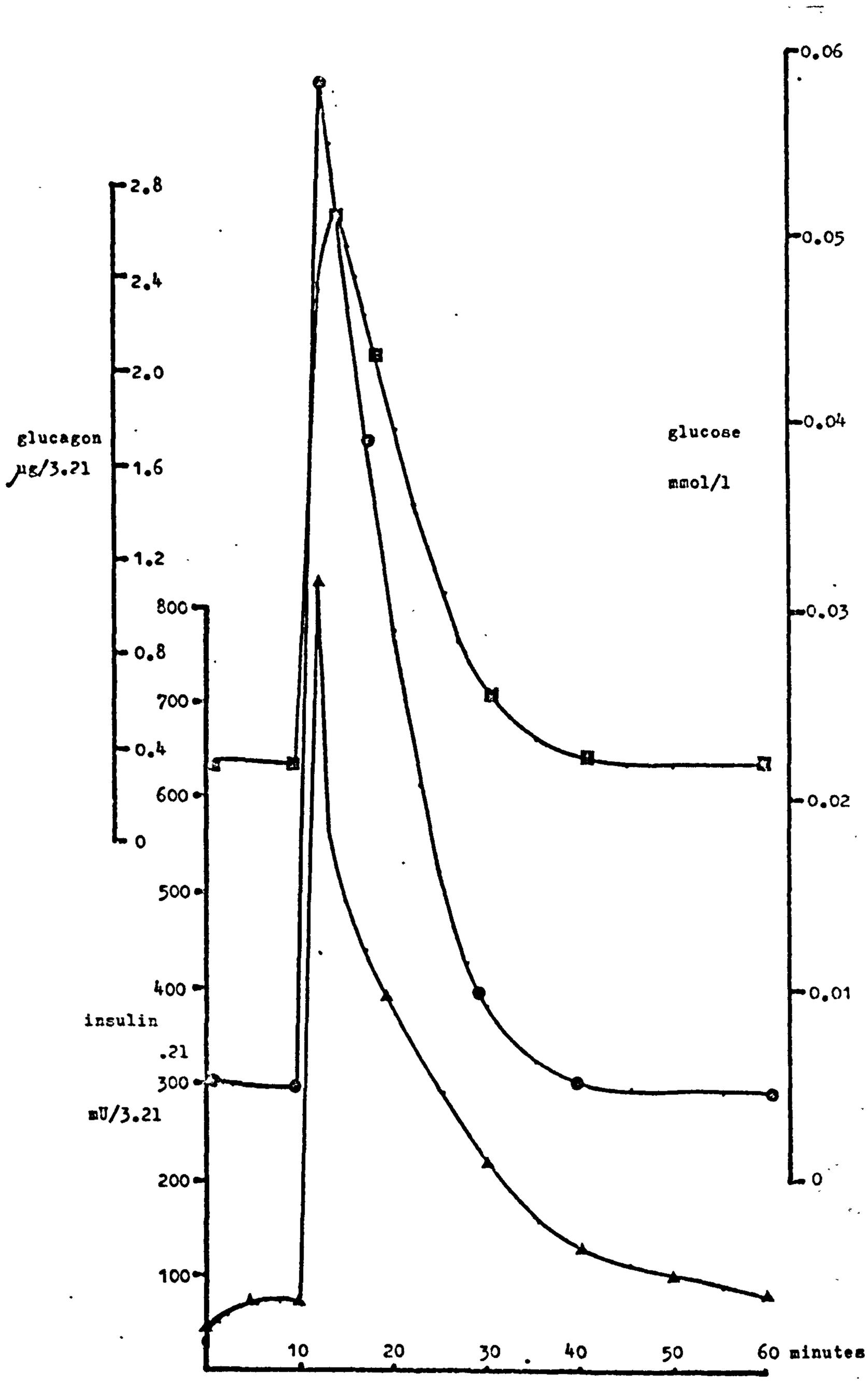


Figure 25 Simulation of intravenous glucose tolerance test. Conditions set as in run 12. There is a good general conformational agreement between the model simulation and typical experimental curves.

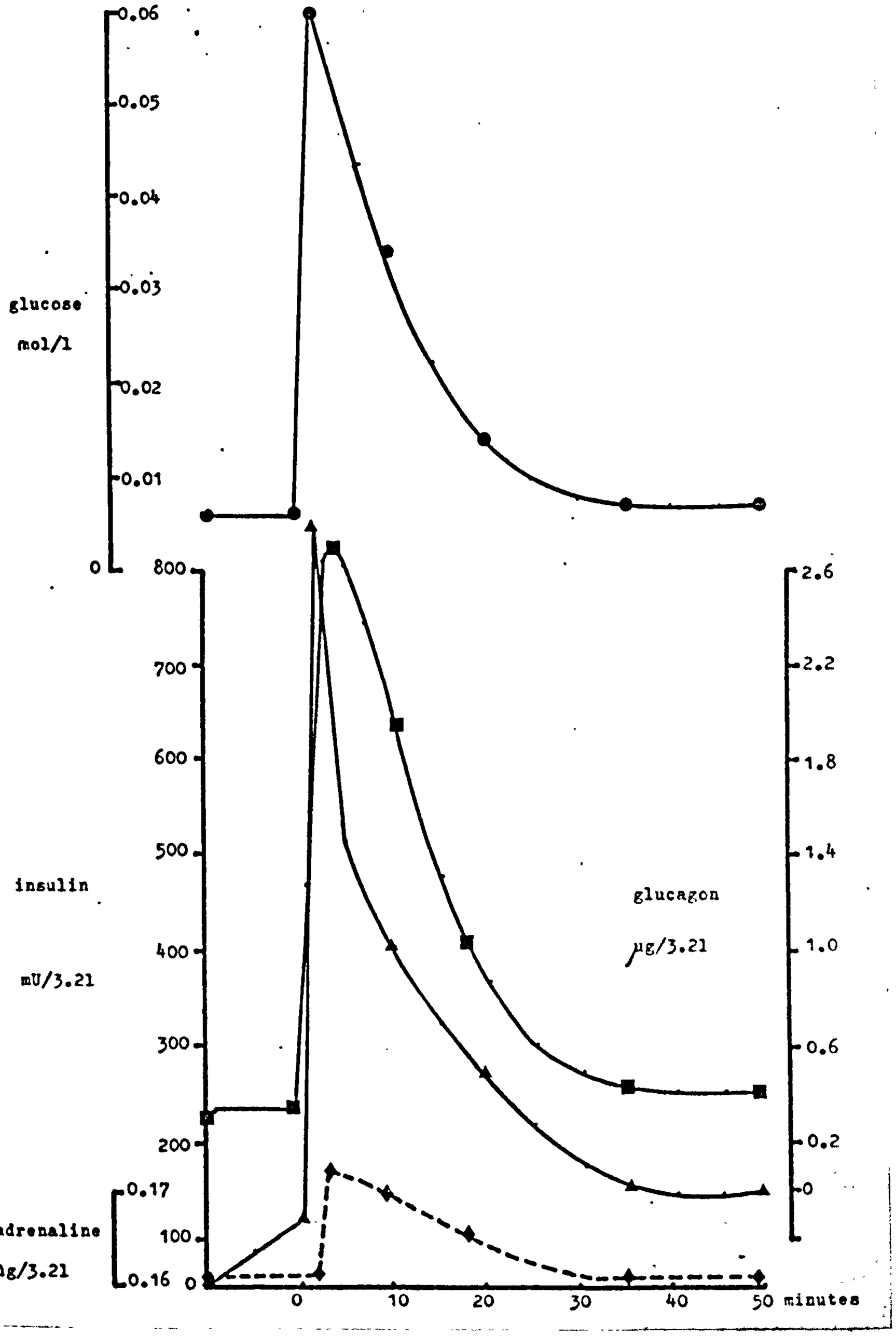


Figure 26 This depicts an unsuccessful attempt to improve upon the previous model by incorporating adrenalin. However, there is an inherent instability in the fasting state.

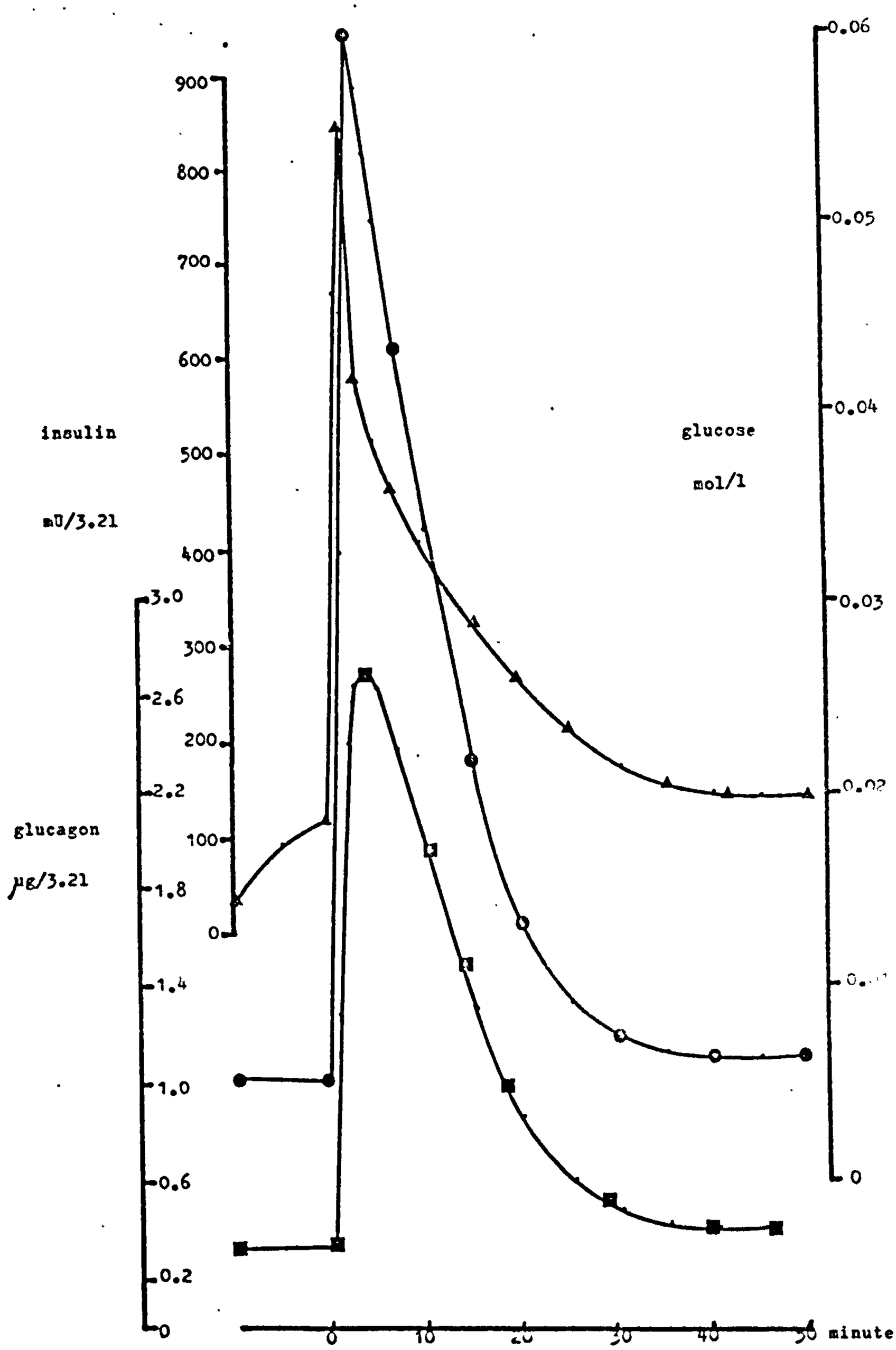


Figure 27 Simulation of continuous glucose infusion. Conditions set as for run 3. Not acceptable.

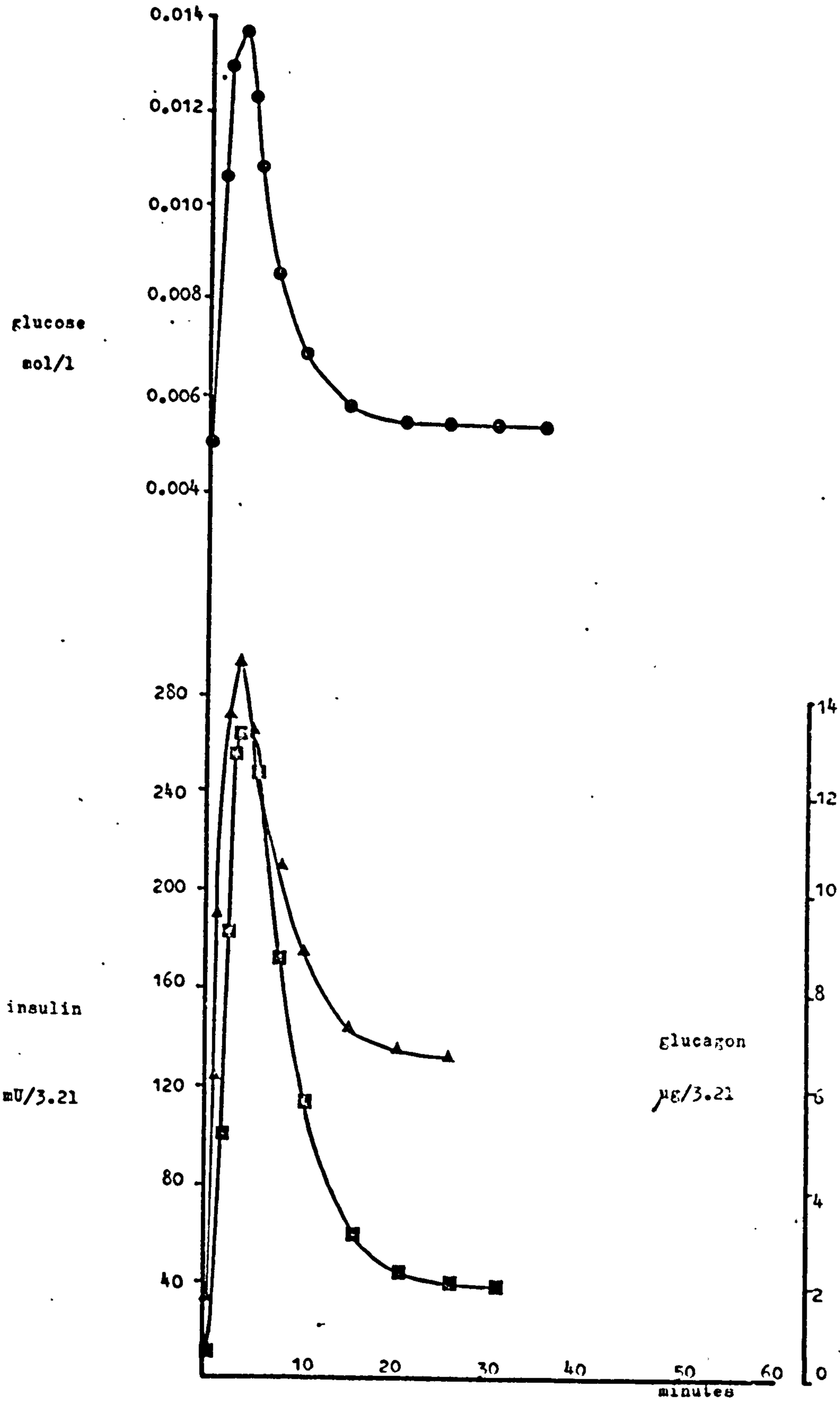


Figure 28 Simulation of continuous glucose infusion. Conditions set as for run 10.

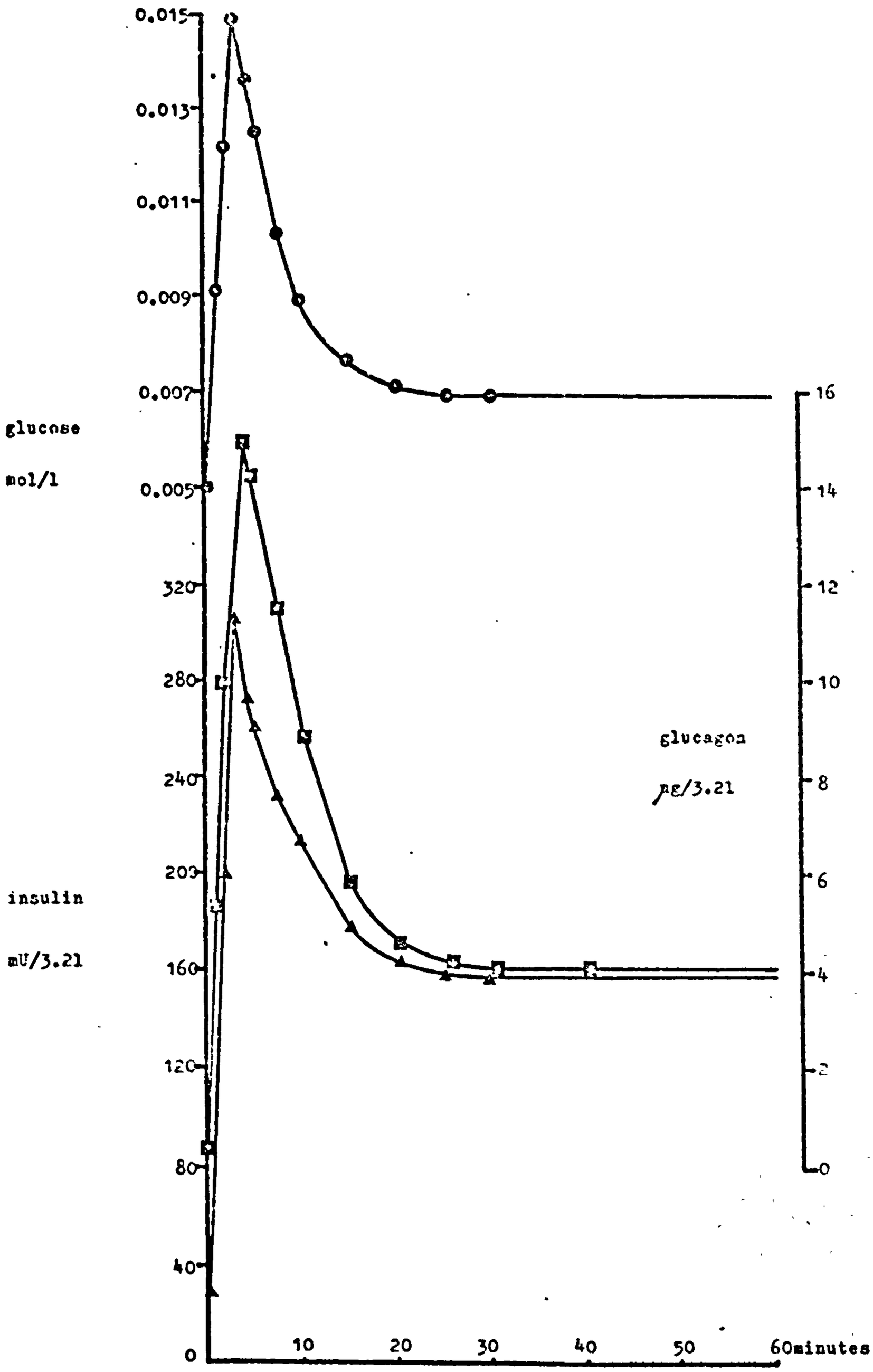


Figure 29 An acceptable simulation of the continuous infusion of glucose. Conditions set as run 12.

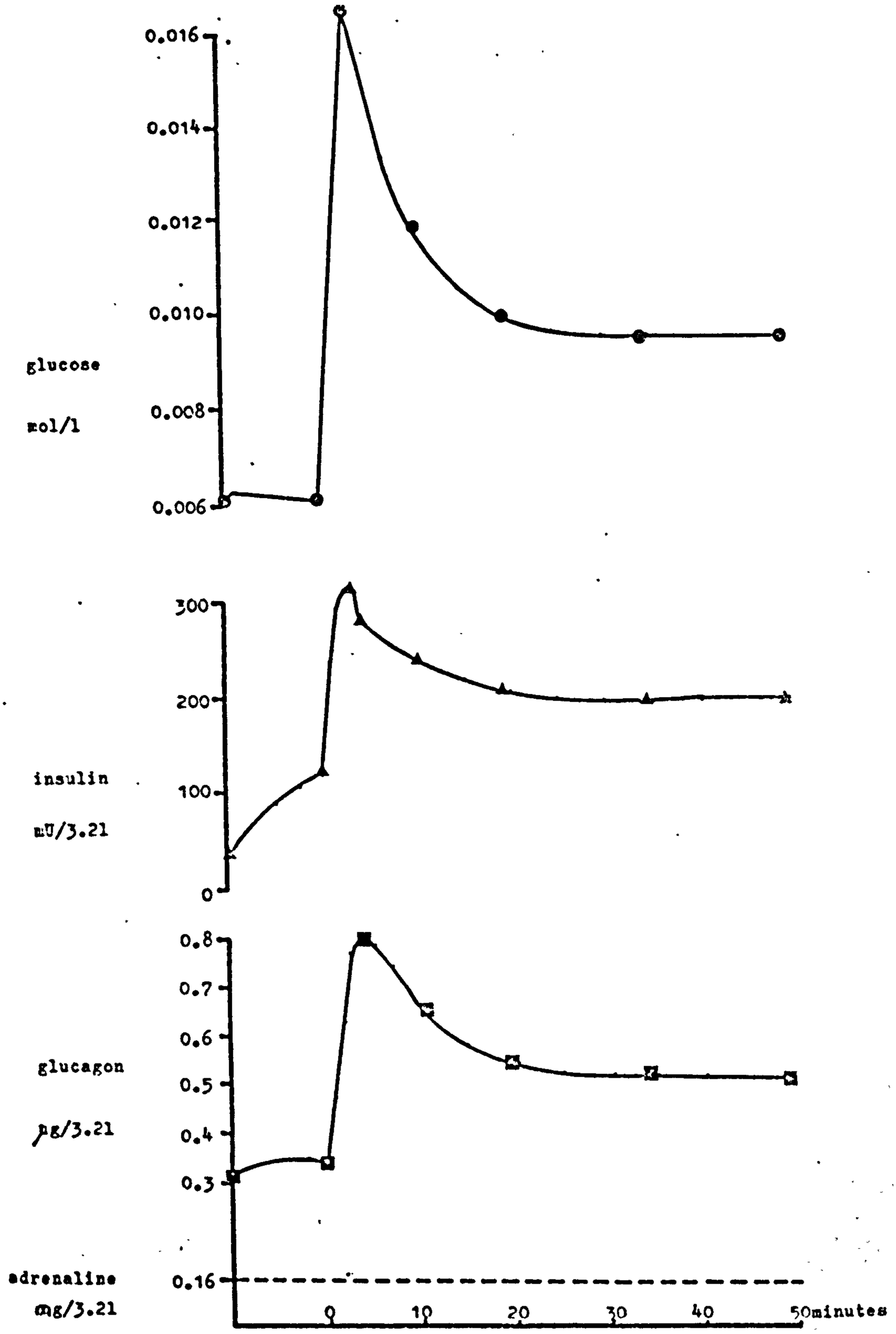


Figure 30 An unsuccessful attempt to include adrenalin in the previous model. However, the fasting plasma glucose concentration and the infusion steady-state are inappropriately high.

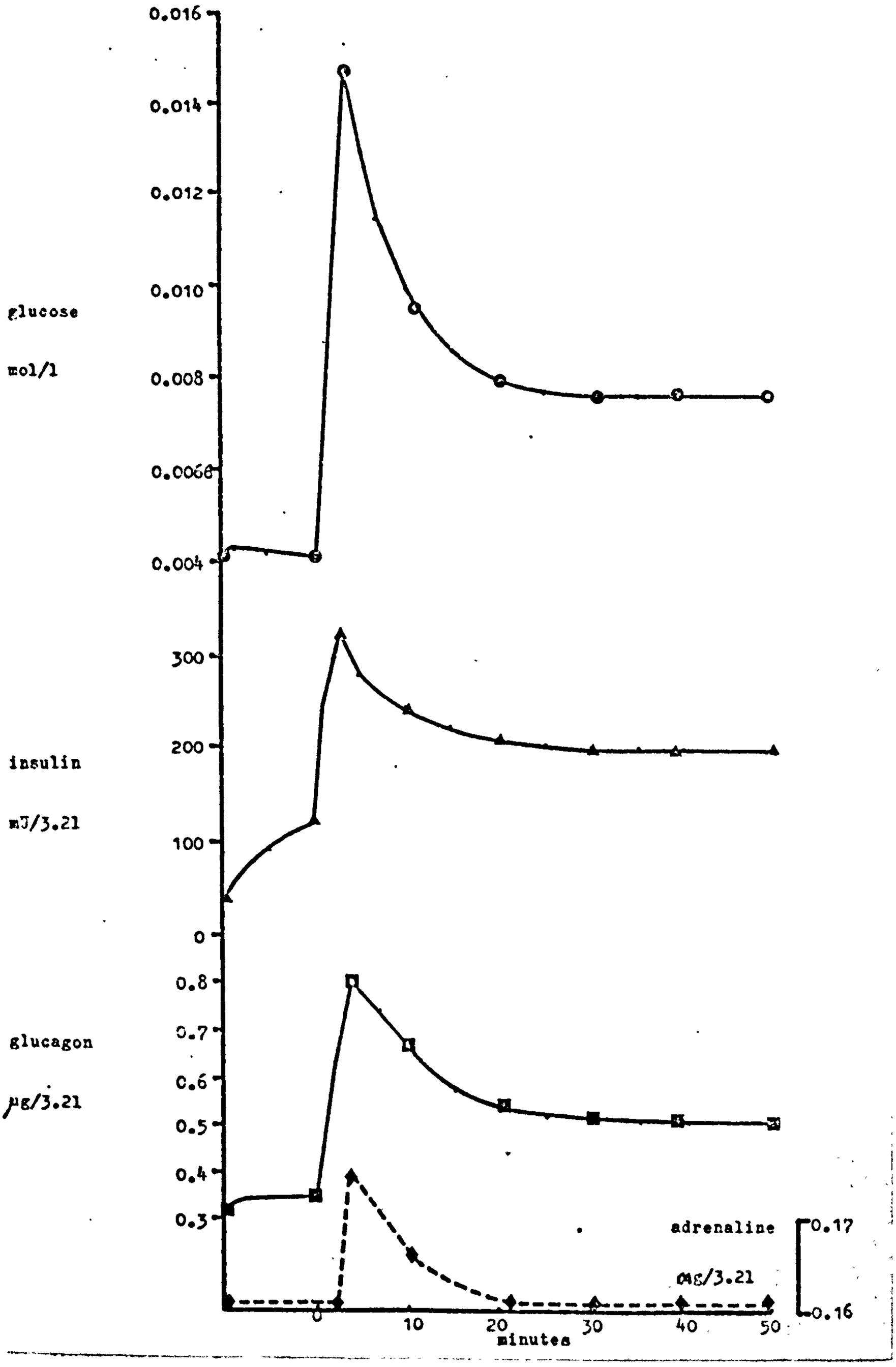


Figure 31 A more successful attempt to incorporate adrenalin. Conditions set as run 16(a).

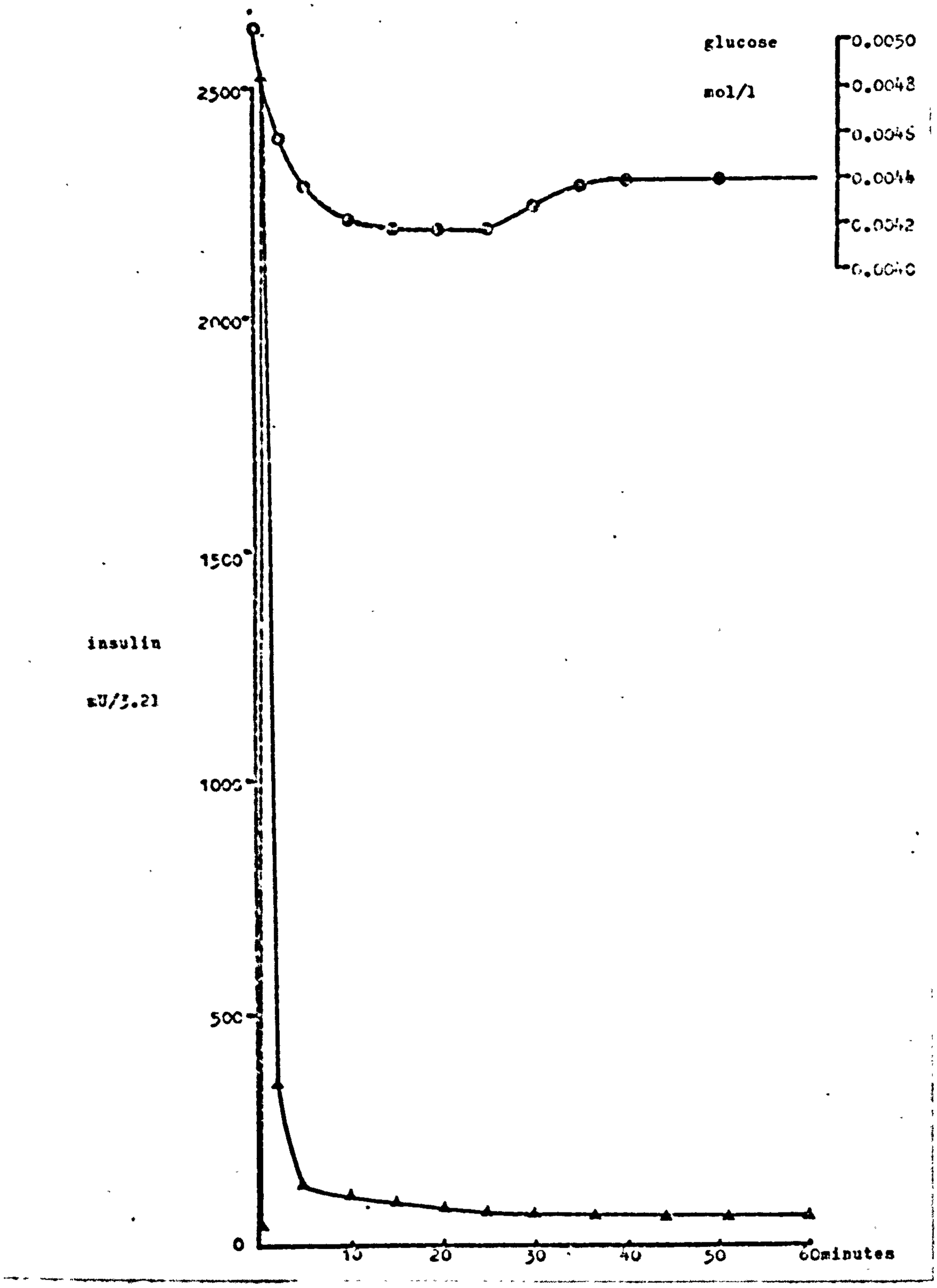


Figure 32 Simulation of insulin injection.
Set for run 1. Not acceptable.

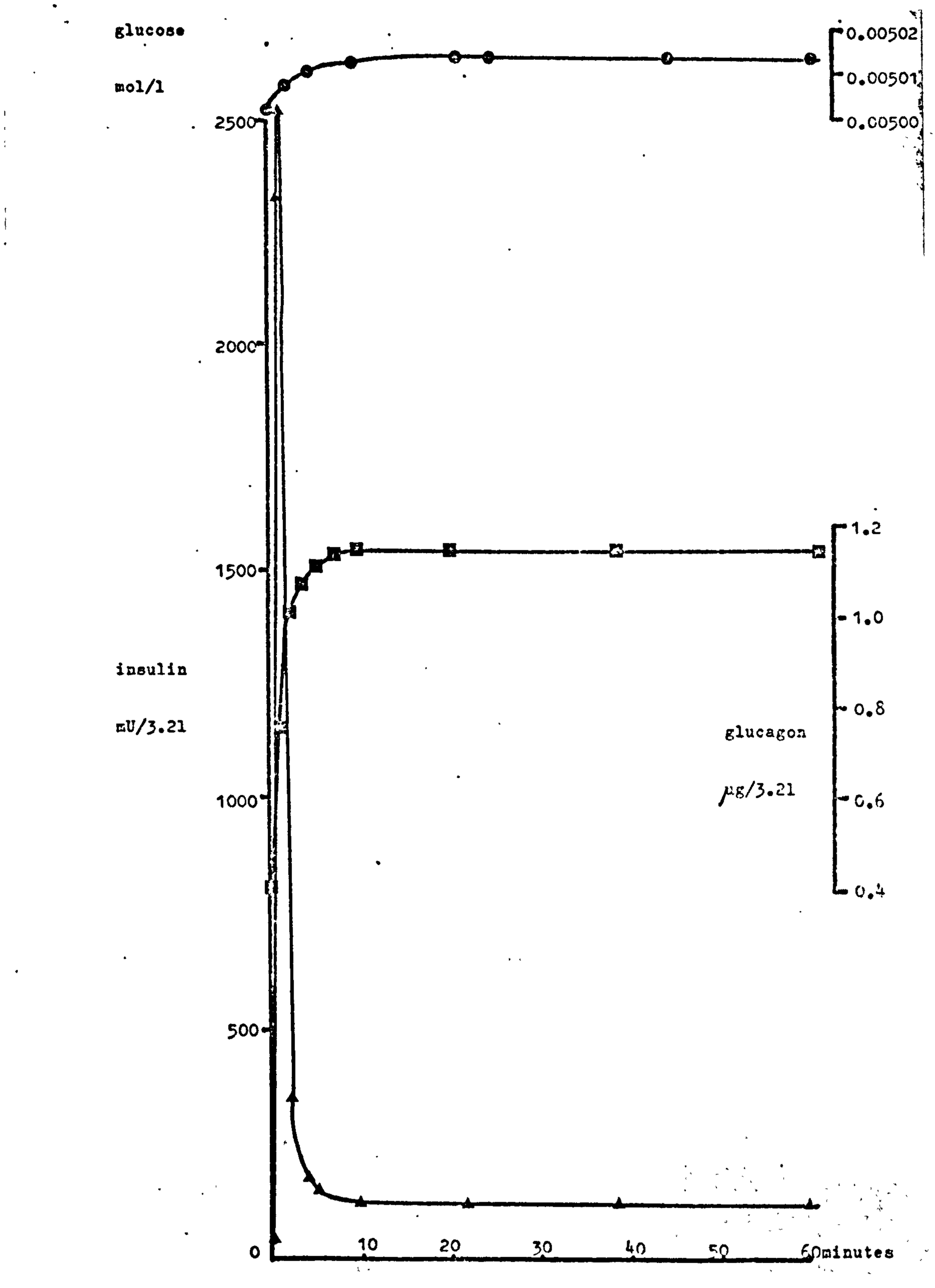


Figure 33 Simulation of insulin injection. Set for run 3. Not acceptable.

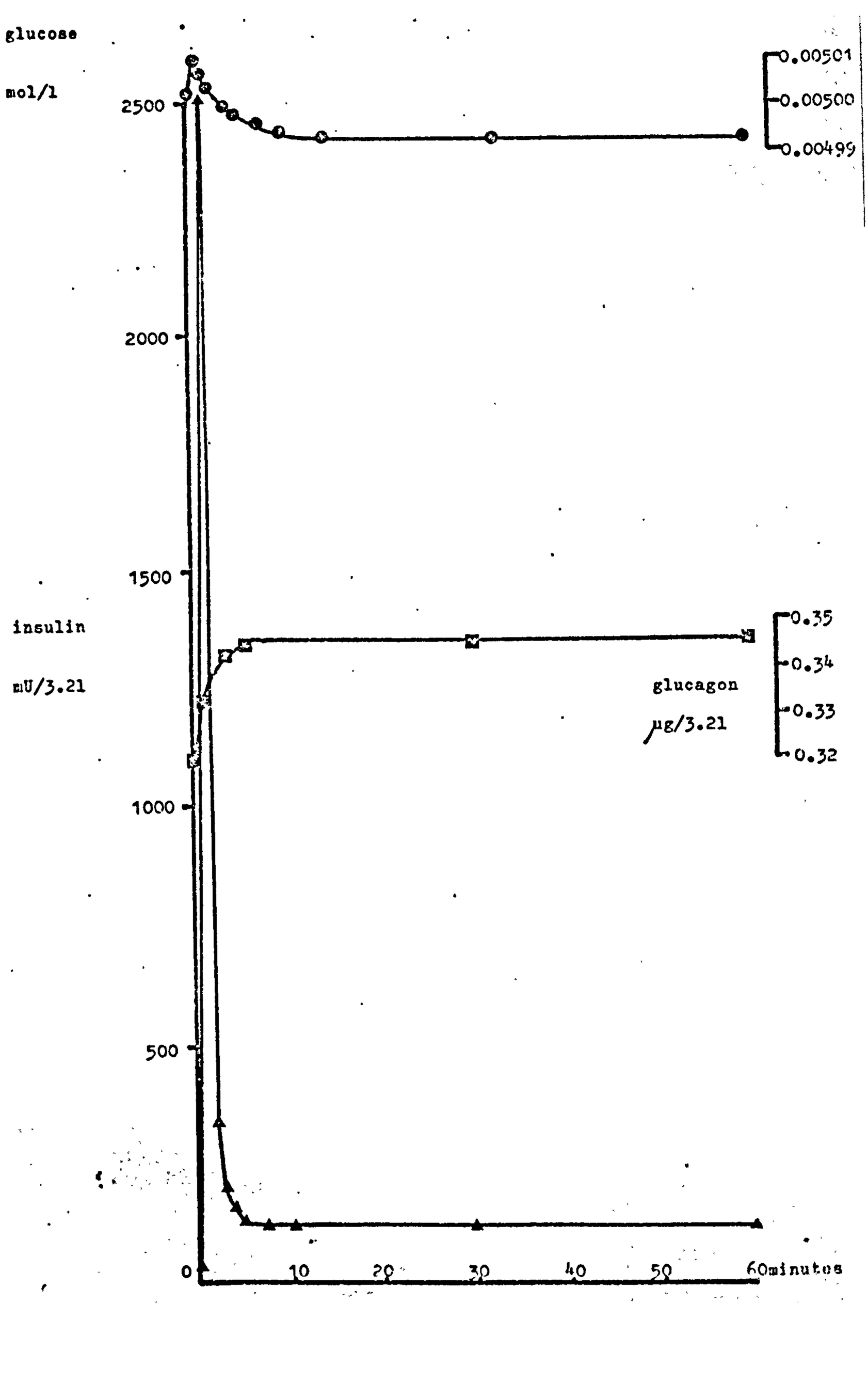


Figure 34 Simulation of insulin injection.
Set for run 7. Not acceptable.

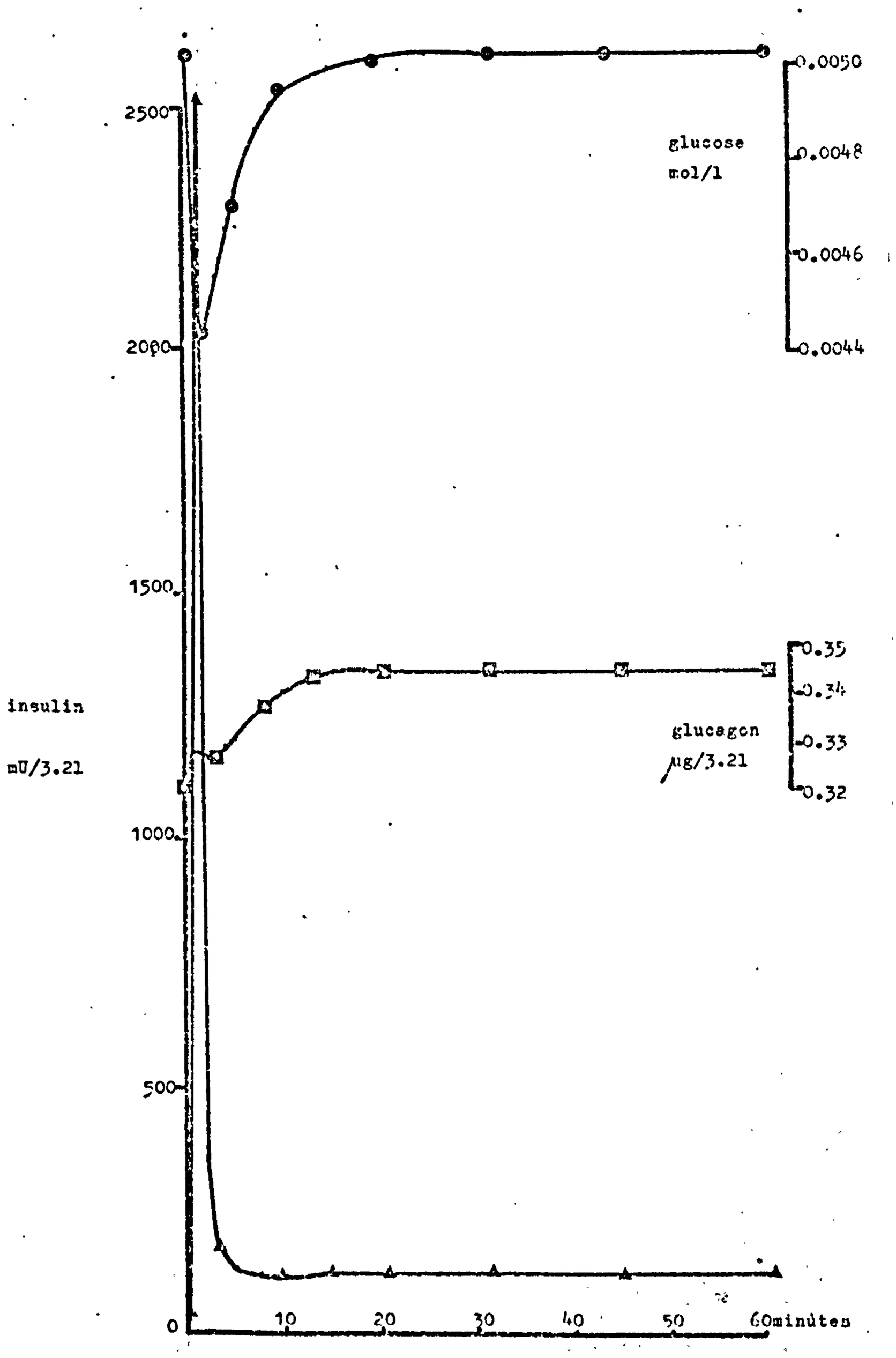


Figure 35 Simulation of insulin injection. Set for run 9. Not acceptable.

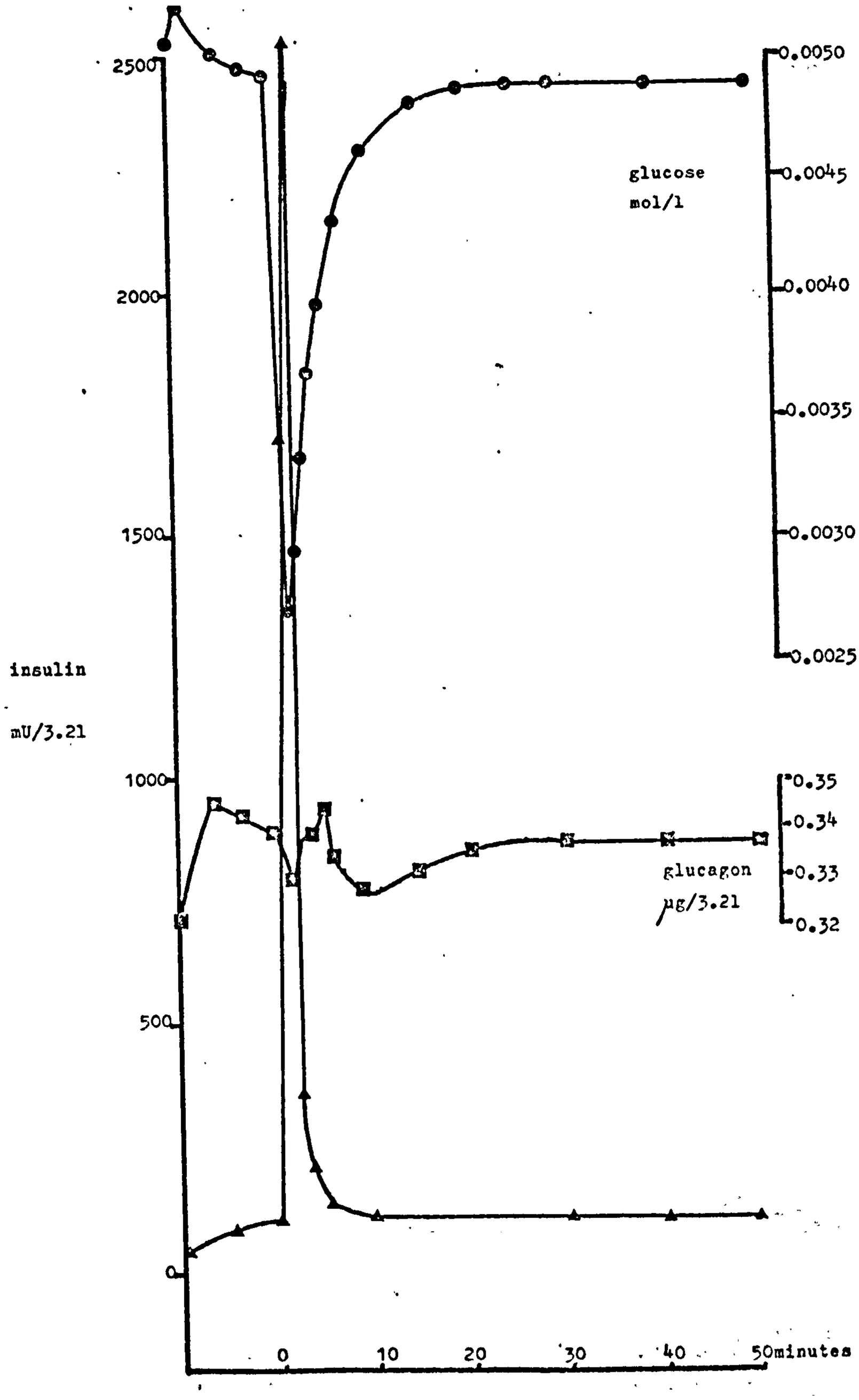


Figure 36 Simulation of insulin injection.
Set for run 12. General conformation acceptable.

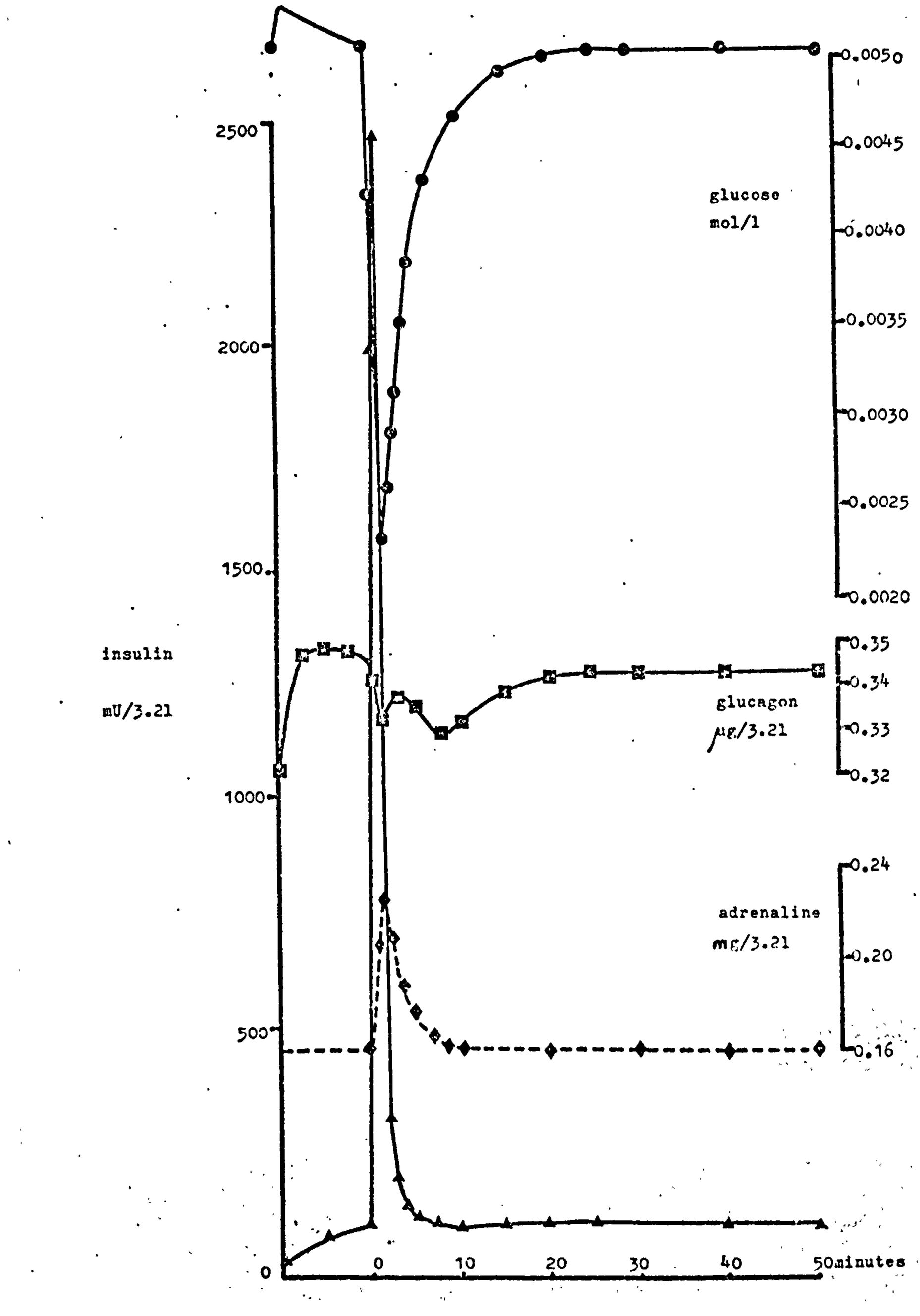


Figure 37 Simulation of insulin injection. Incorporation of adrenalin has improved the simulation. Set for run 16(a).

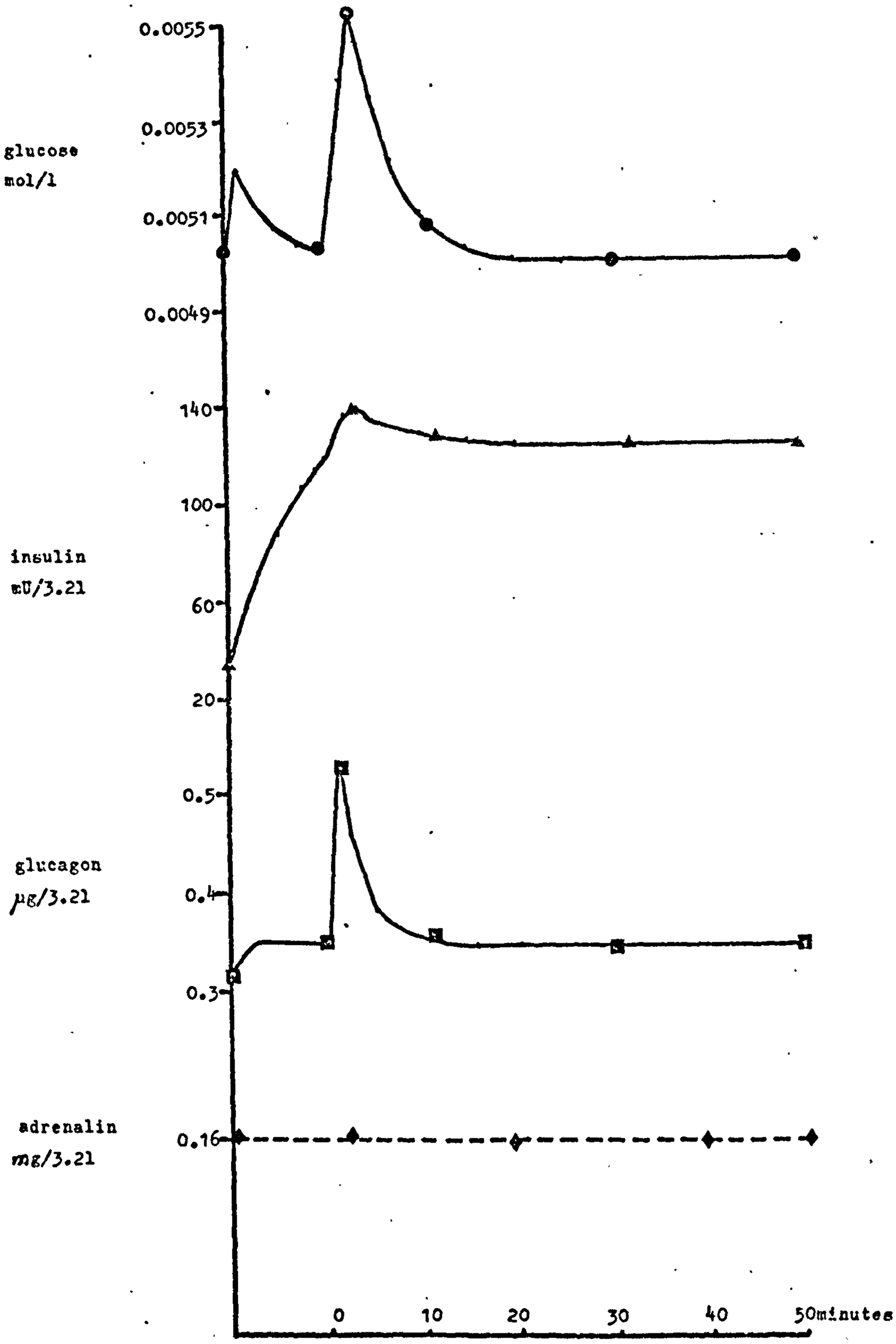


Figure 38 Simulation of glucagon injection.
Set for run 17. Not acceptable.

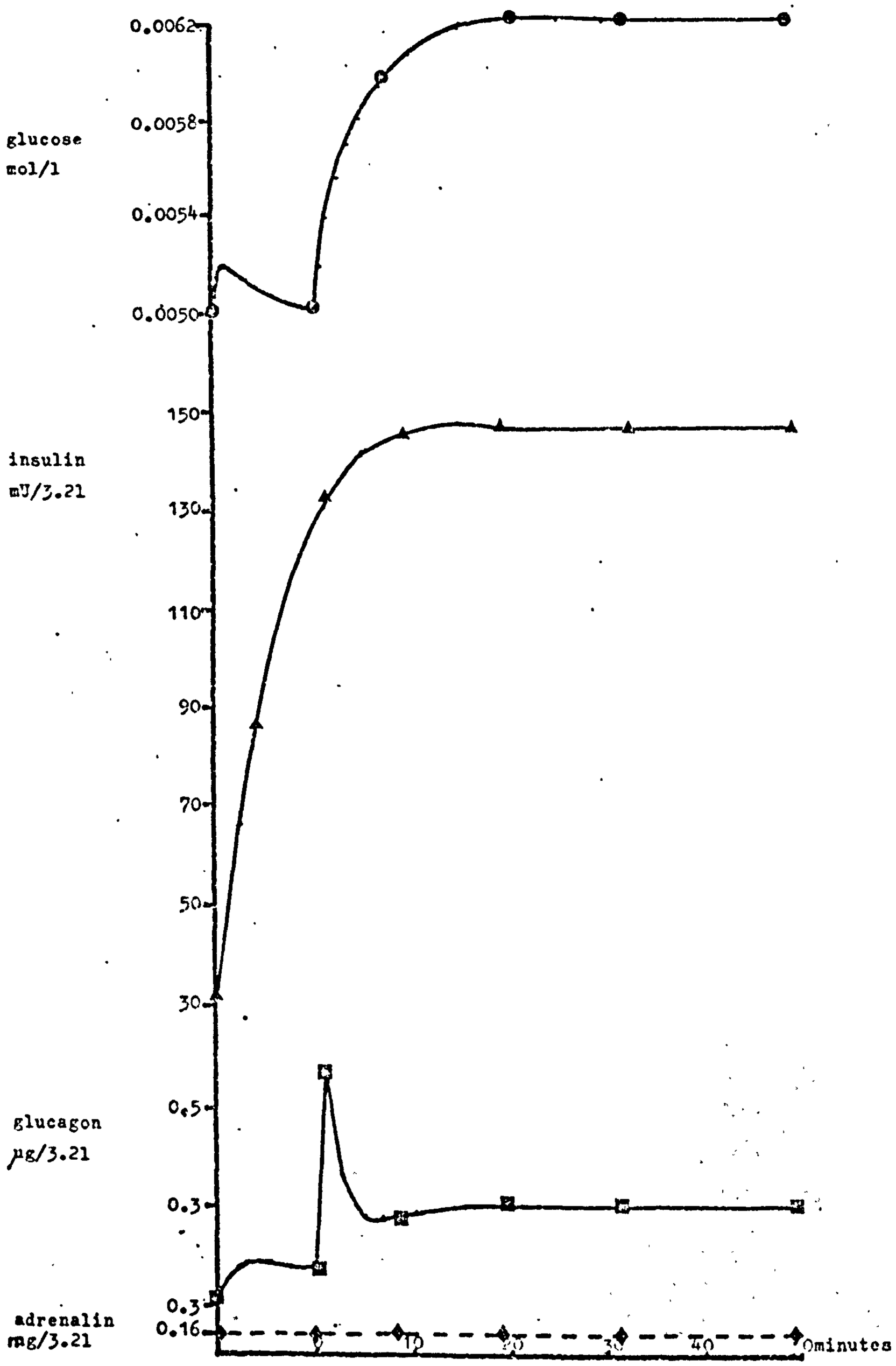


Figure 39 Simulation of glucagon injection.
Set for run 20. Not acceptable.

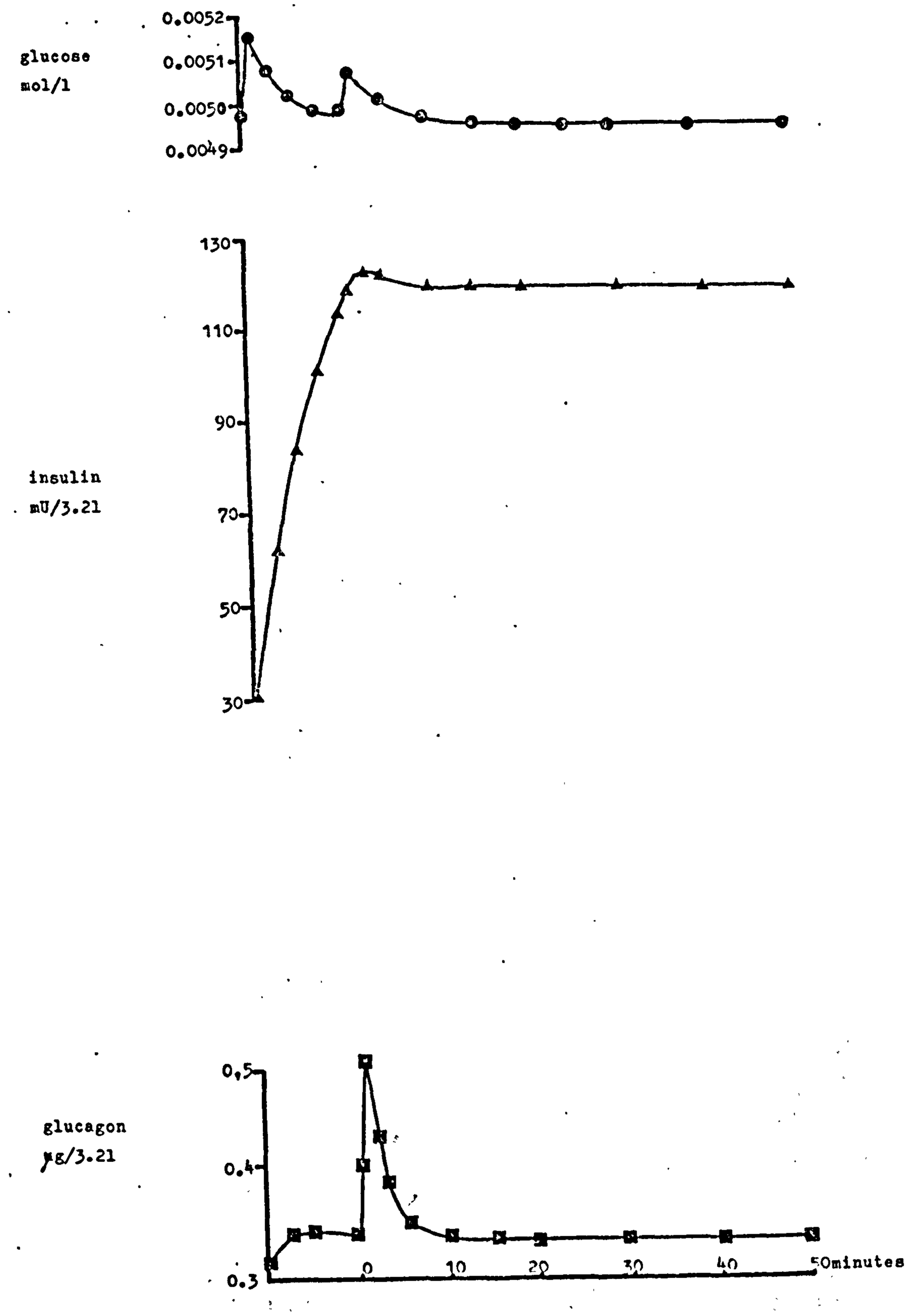


Figure 40 Simulation of glucagon injection.
Set for run 18. Not acceptable.

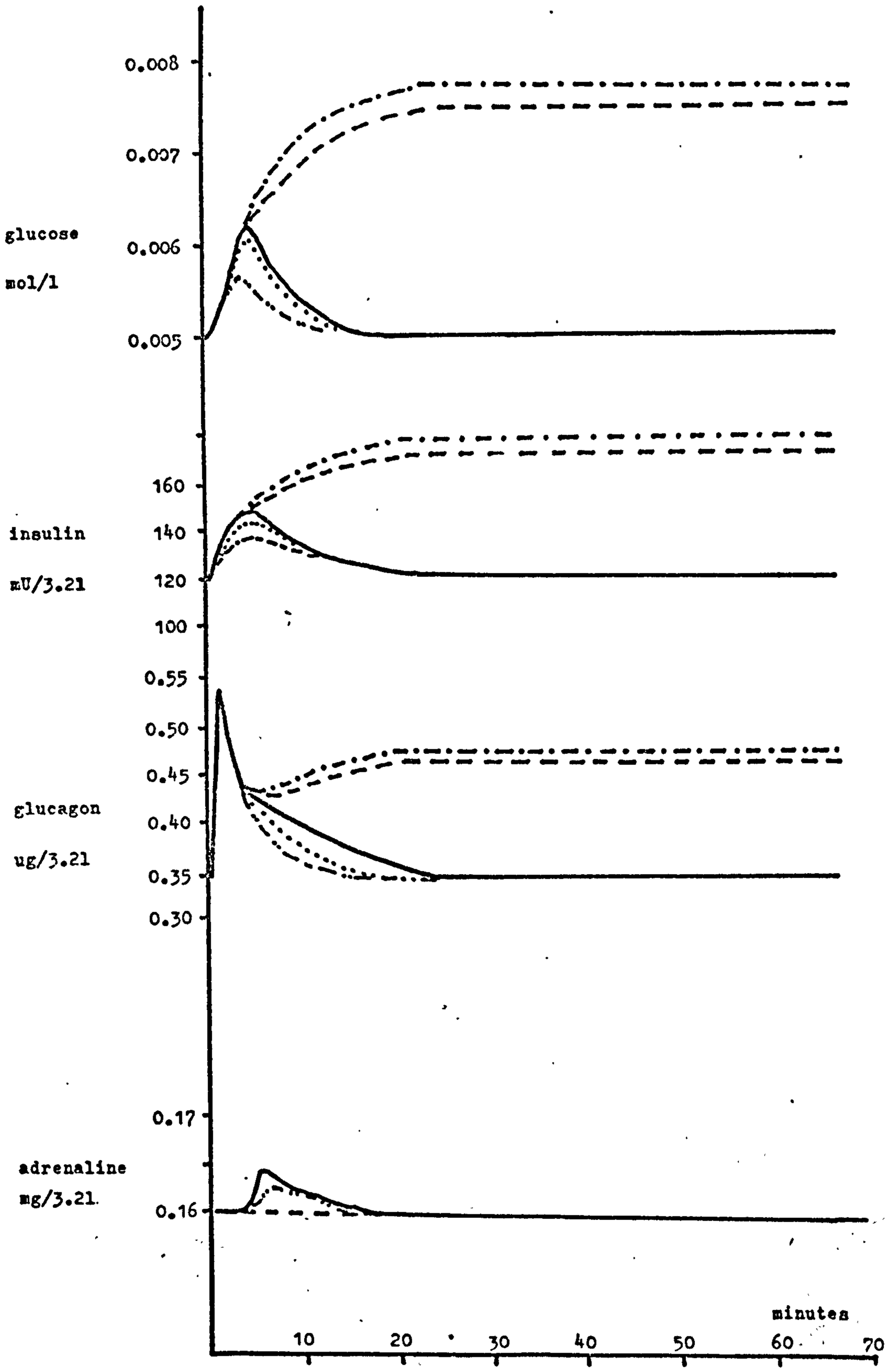

glucose
mol/l
0.008
0.007
0.006
0.005
insulin
mU/3.21
160
140
120
100
glucagon
ug/3.21
0.55
0.50
0.45
0.40
0.35
0.30
adrenaline
mg/3.21.
0.17
0.16
minutes
10
20
30
40
50
60
70

Figure 44 Simulation of the model response to a glucose infusion but with the activity of glucose-6-phosphatase reduced by 30% of its normal value. Note fasting hypoglycaemia and under-shooting before the new steady-state is gained.

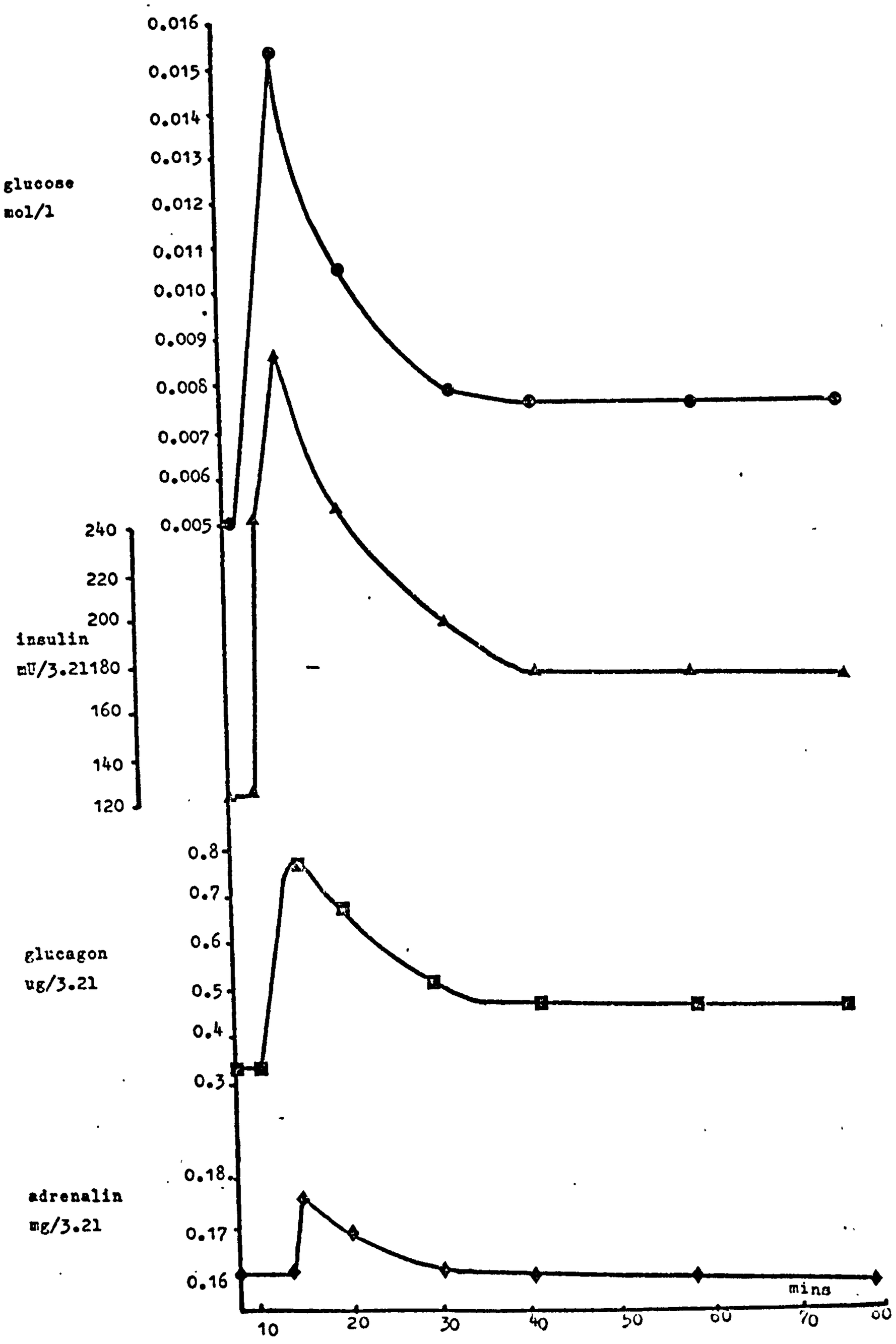


Figure 42 Simulation of glucose infusion but with the phosphorylase reaction deleted from the model. Note there is no change in the fasting plasma glucose concentration.

Figure 45 Model response following intravenous glucose injection, but with reduction of glucose-6-phosphatase activity. Note the switching of glucose to a new steady-state level, the multiphasic adrenalin response, and the threshold level of insulin.

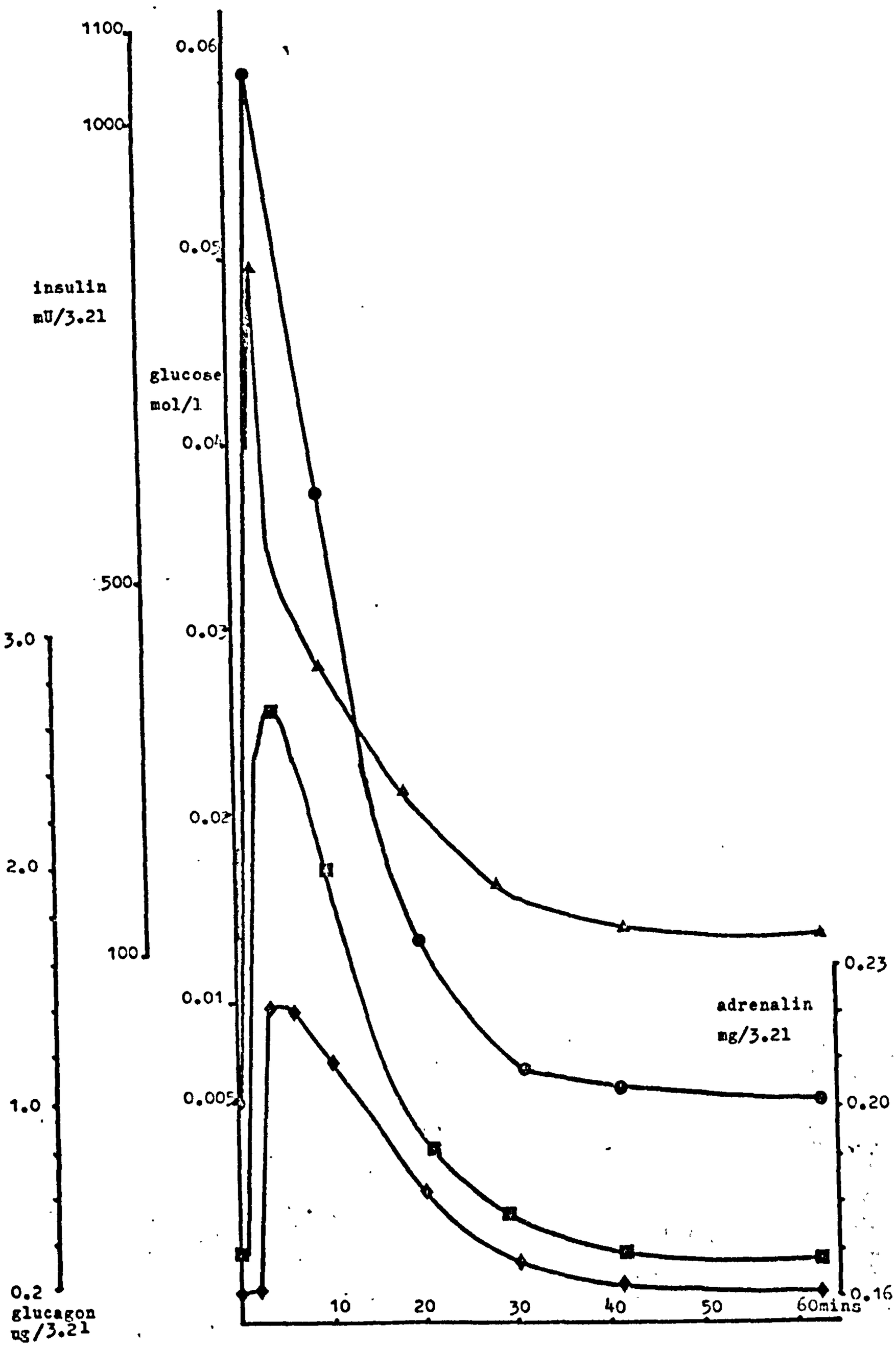


Figure 43 Simulation of the model response to intravenous glucose injection following deletion of the phosphorylase reaction from the model.

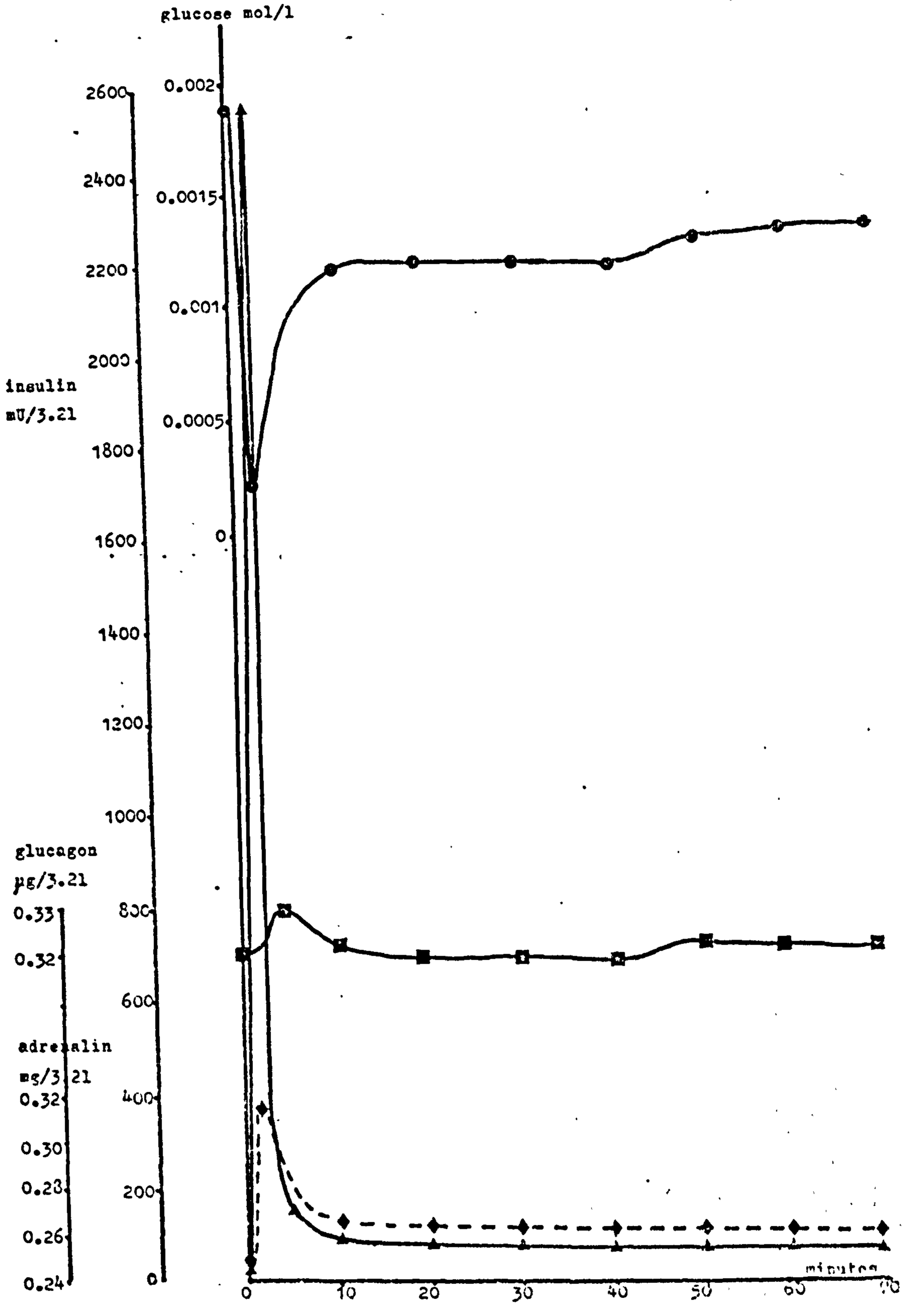


Figure 46 Model response, following insulin injection but with glucose-6-phosphatase activity reduced 30%. There is a marked hypoglycaemia with no evidence of returning to the fasting concentration.

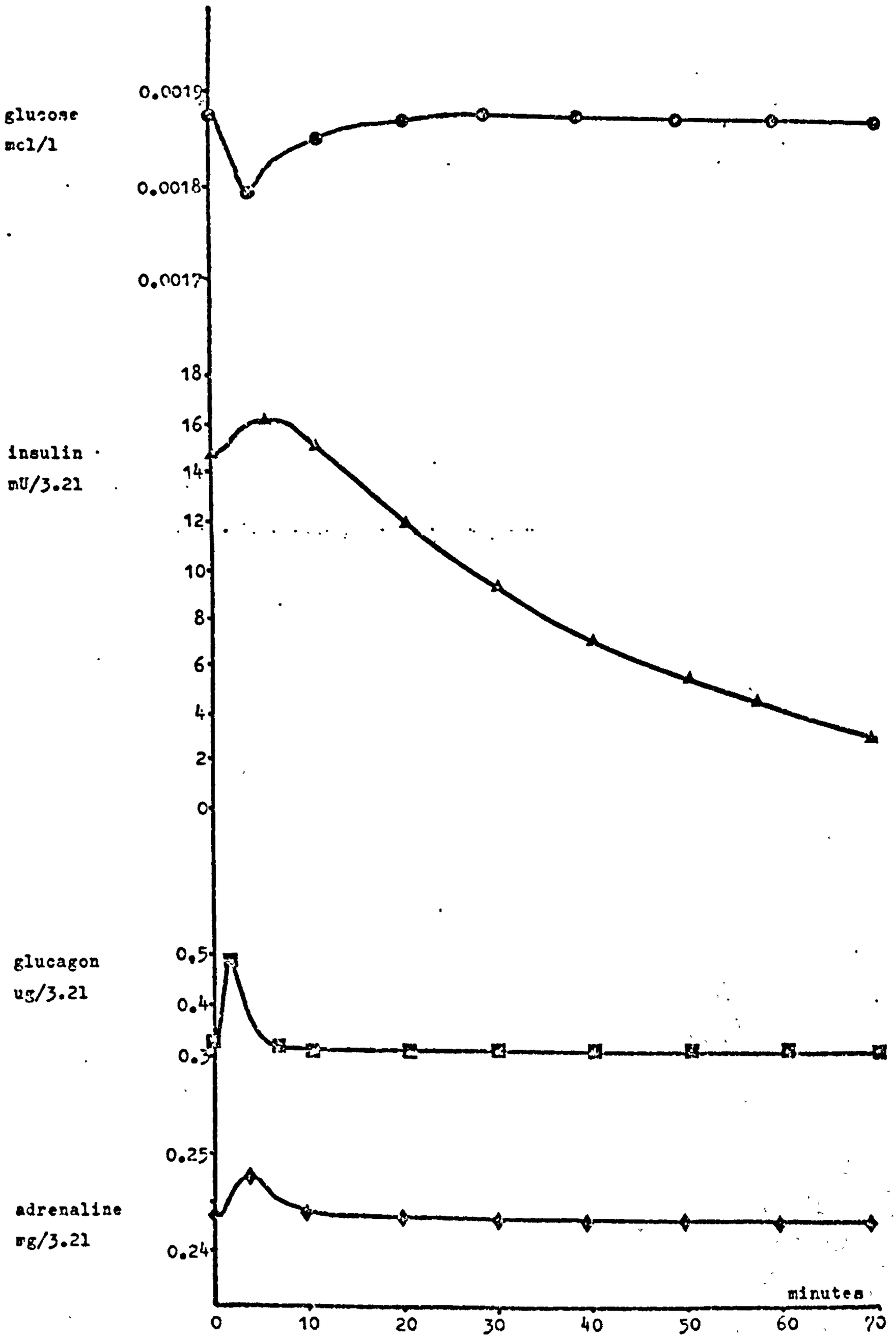


Figure 47 Result of model response to glucagon injection when glucose-6-phosphatase activity was reduced 30%. Note transient depression of plasma glucose and switching of insulin to a new level.

Figure 48 Model response to glucose infusion when the glucagon threshold is varied. Curves 1, 2, and 3 represent threshold values of 0.20, 0.39 and 0.60 respectively. Lowering the threshold results in higher glucose steady-state values, probably by increasing hepatic glucose output.

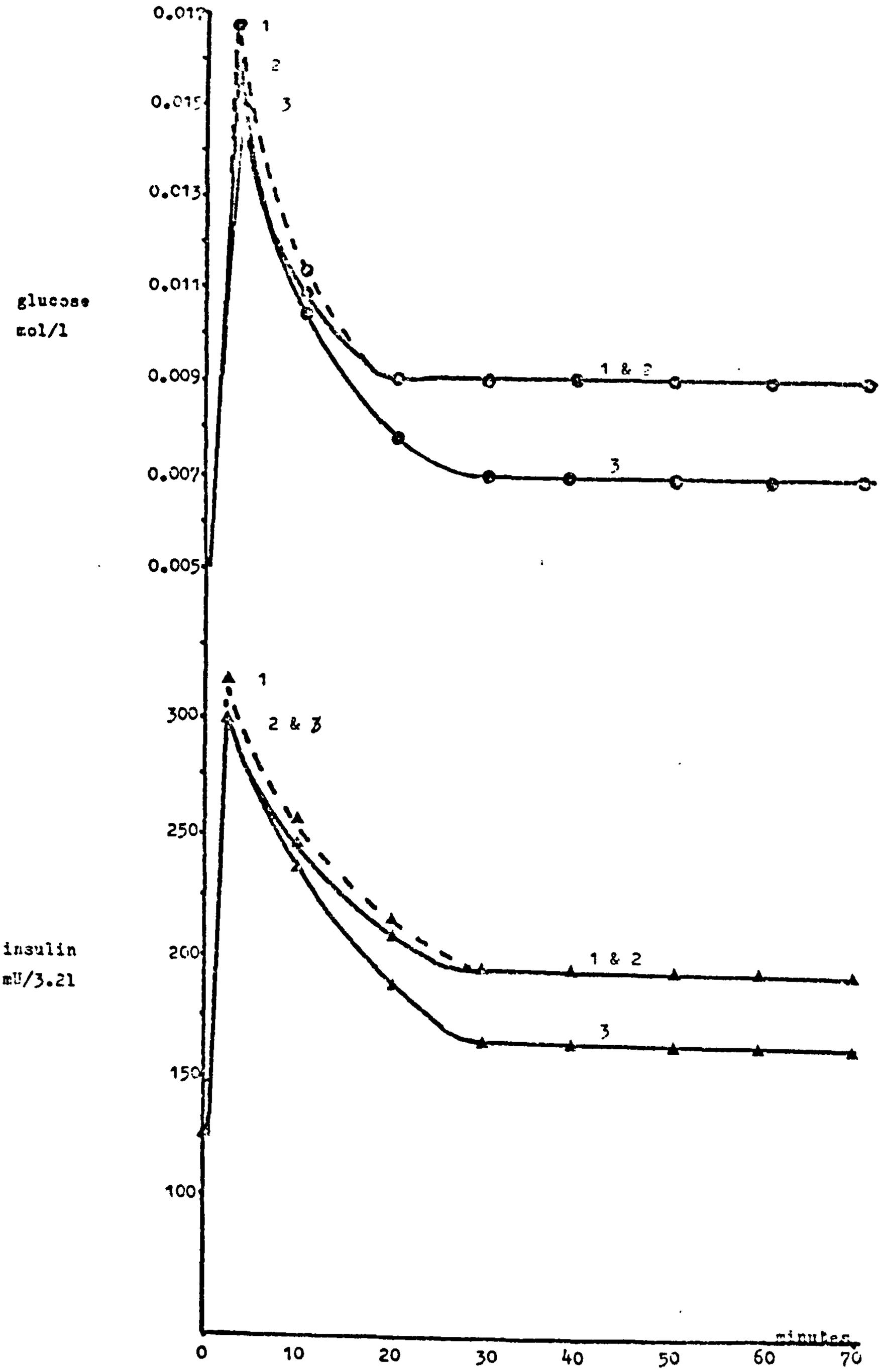
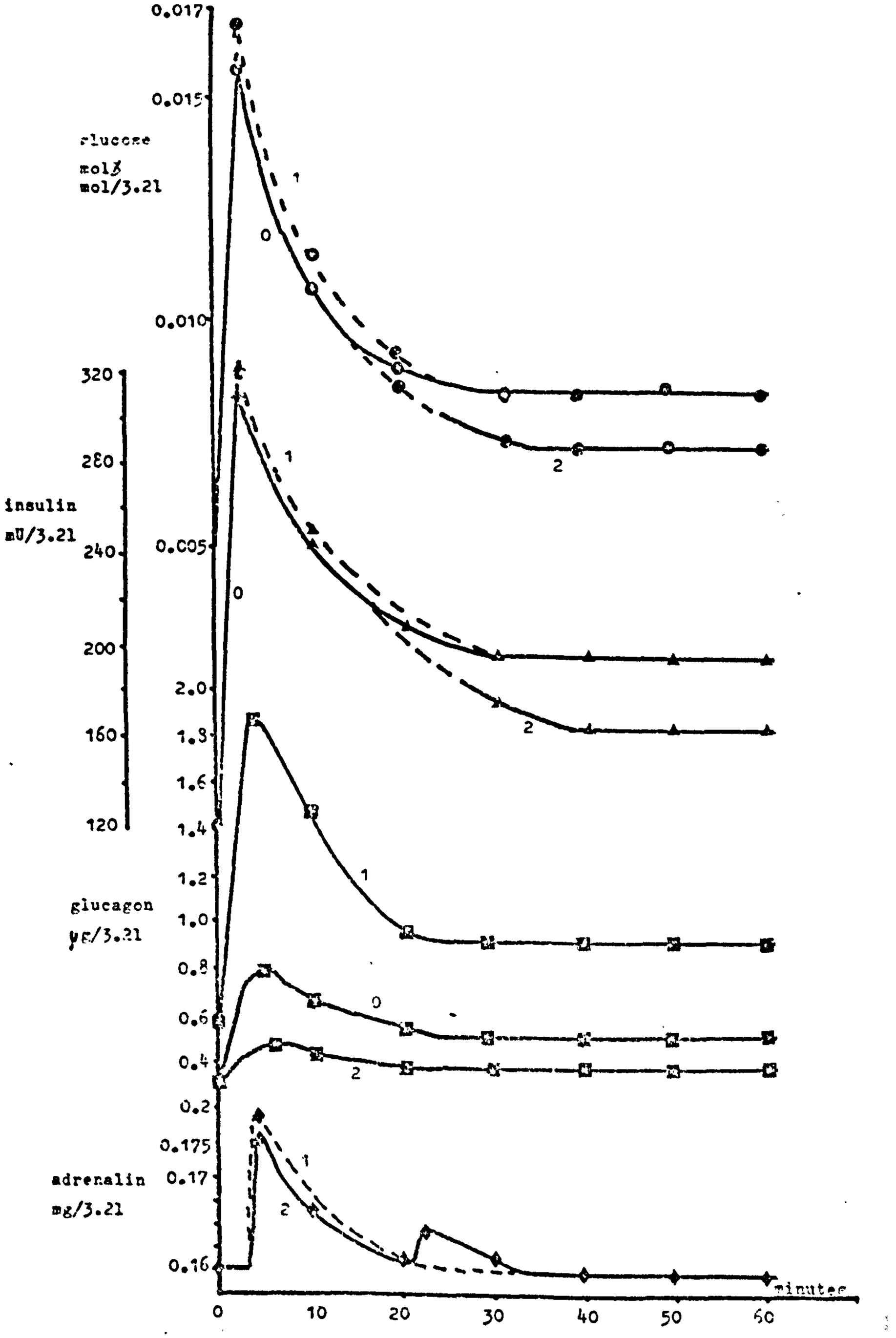

glucose
mol/l
0.017
0.015
0.013
0.011
0.009
0.007
0.005
1
2
3
1 & 2
3
insulin
mU/3.21
300
250
200
150
100
1
2 & 3
1 & 2
3
0
10
20
30
40
50
60
70
minutes

Figure 49 Model response to glucose infusion but with sensitivity of the glucagon controller varied. Curves 0, 1 and 2 represent values of 30, 90 and 10 in the controller equation. The latter value appears to be optimal. Note the multi-phasic adrenalin response.


0.017
0.015
0.010
0.005
glucose
mol/3.21
mol
1
0
2
320
280
240
200
160
120
insulin
mU/3.21
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
glucagon
µg/3.21
0.175
0.17
0.16
adrenalin
mg/3.21
minutes
0
10
20
30
40
50
60

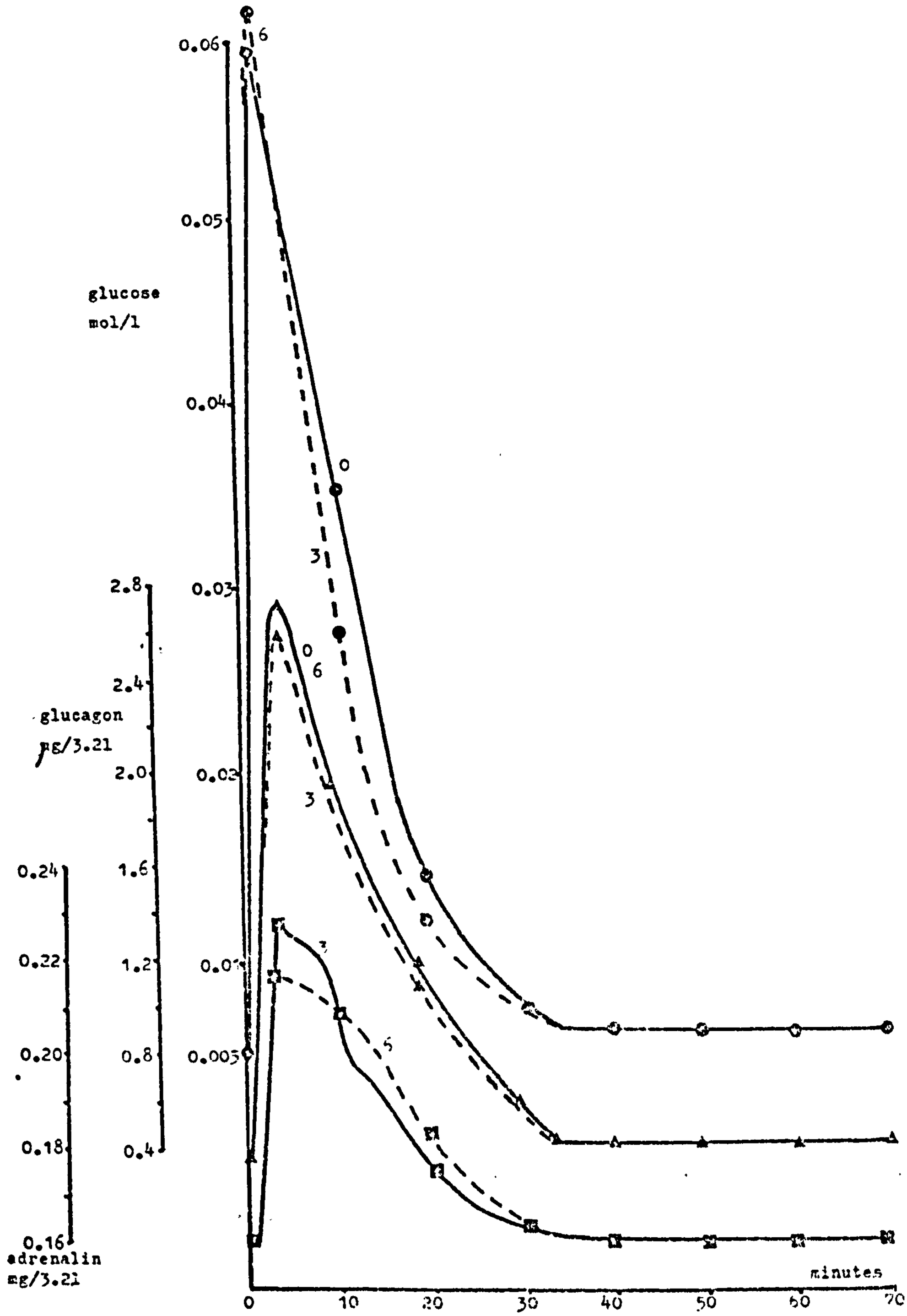


Figure 50 Simulation of model response to glucose injection but with the insulin sensitivity element varied from the theoretical optimum (0).

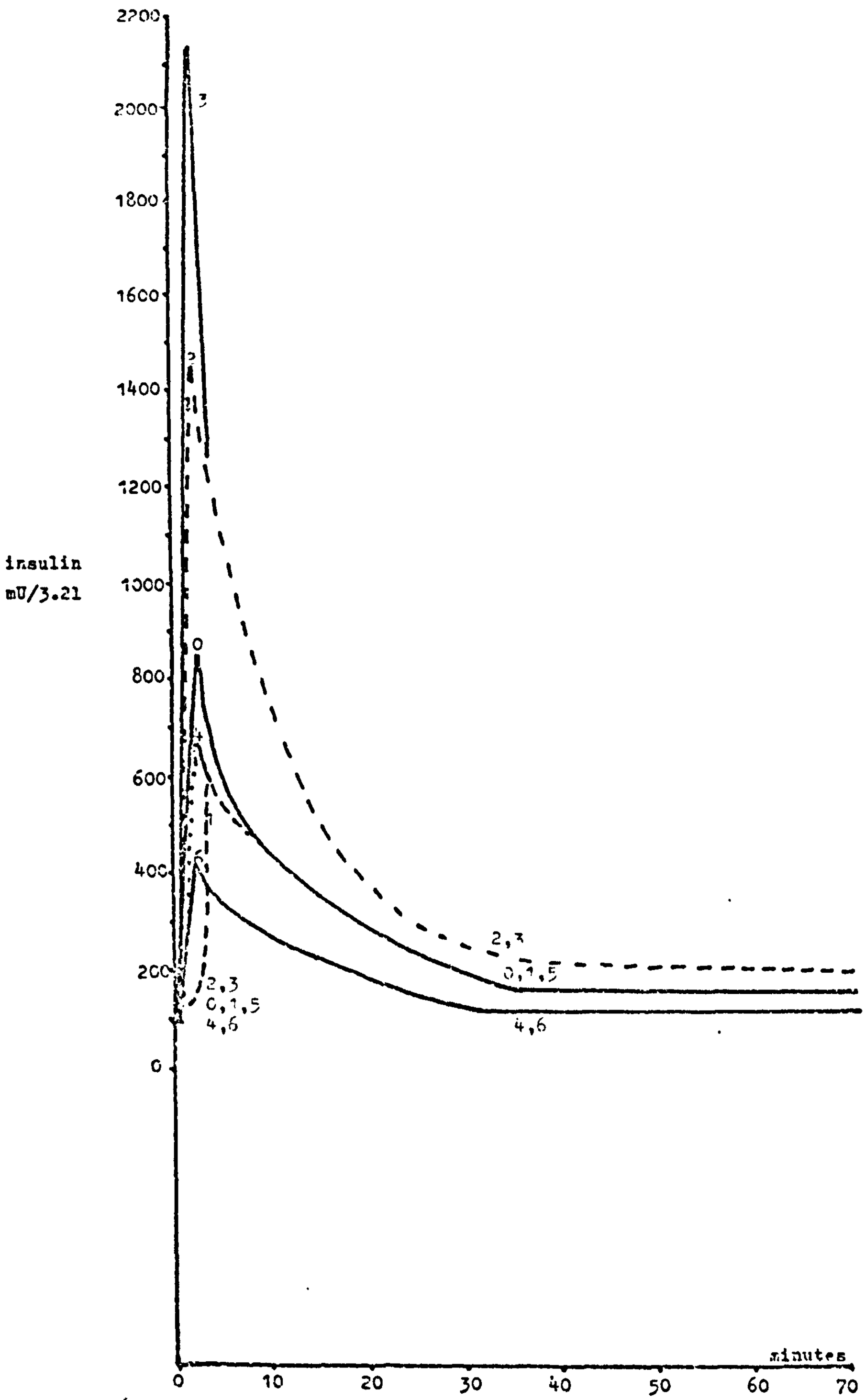


Figure 51 The insulin levels from the system depicted in the previous figure.

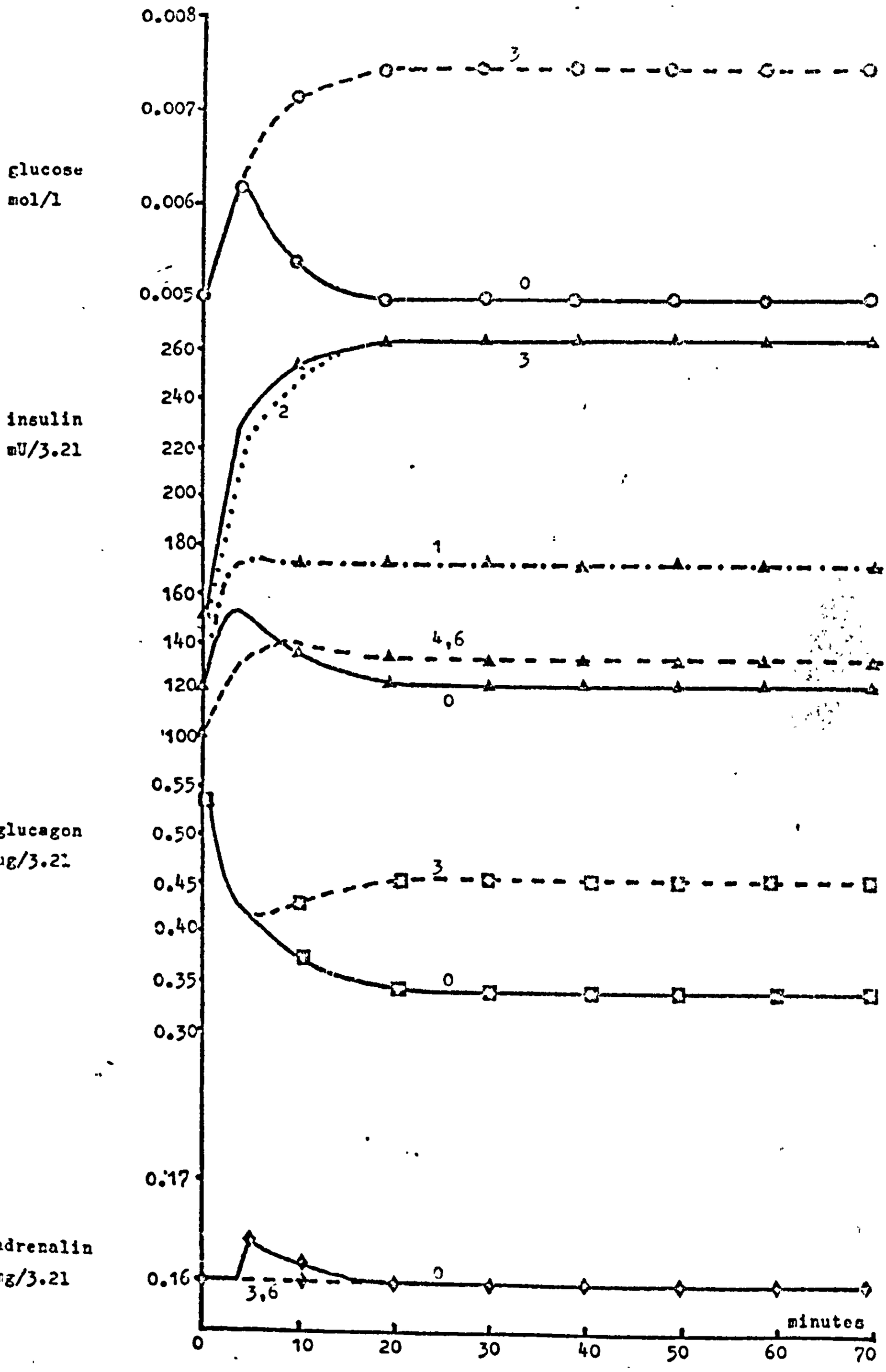


Figure 52 Model response to glucagon injection but with the insulin sensitivity varied as previously.

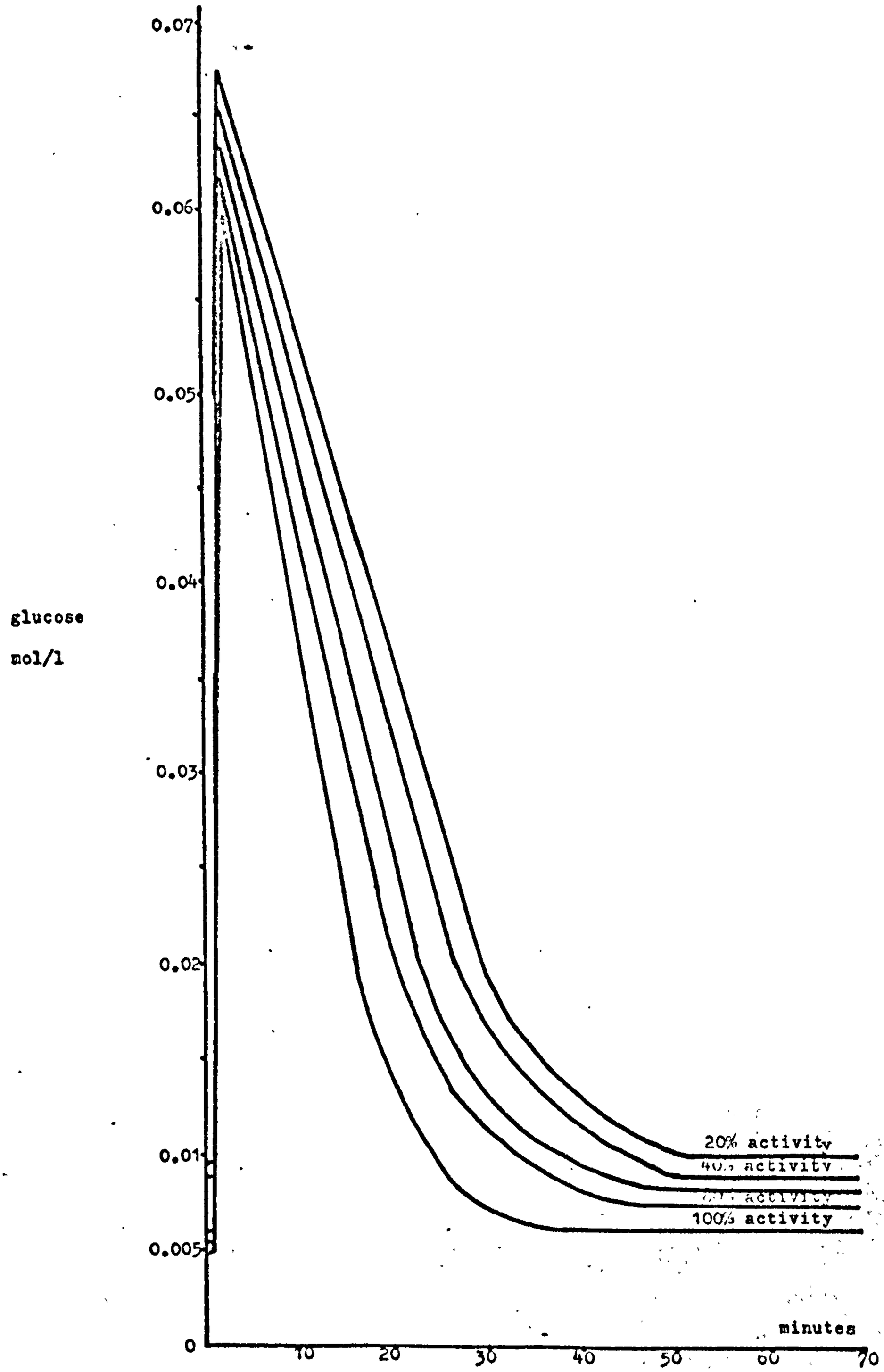


Figure 53 Model response to glucose injection but with progressive reduction of peripheral hexokinase activity.

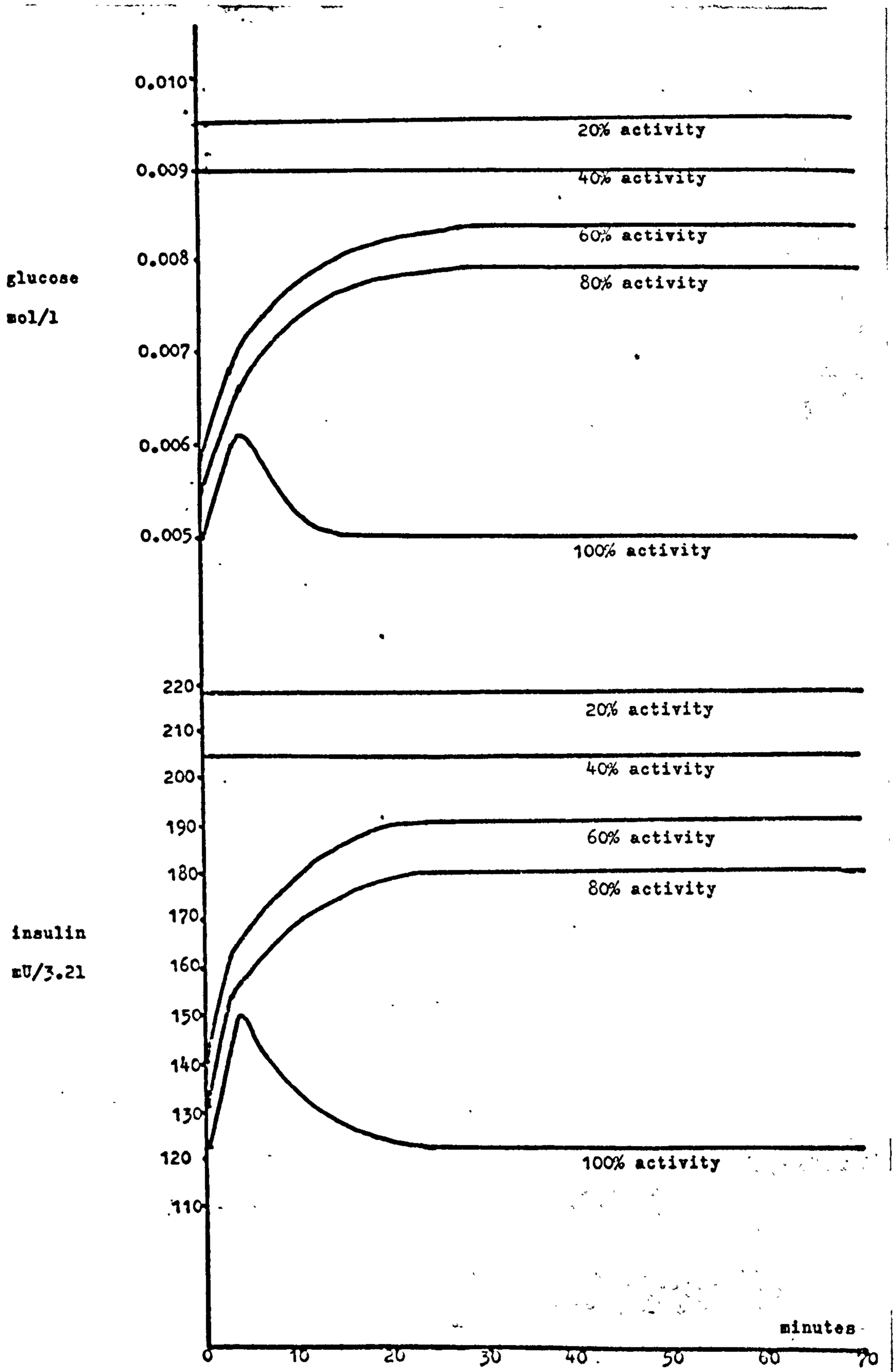


Figure 54 Model response to glucagon injection but with progressive reduction of peripheral hexokinase activity.

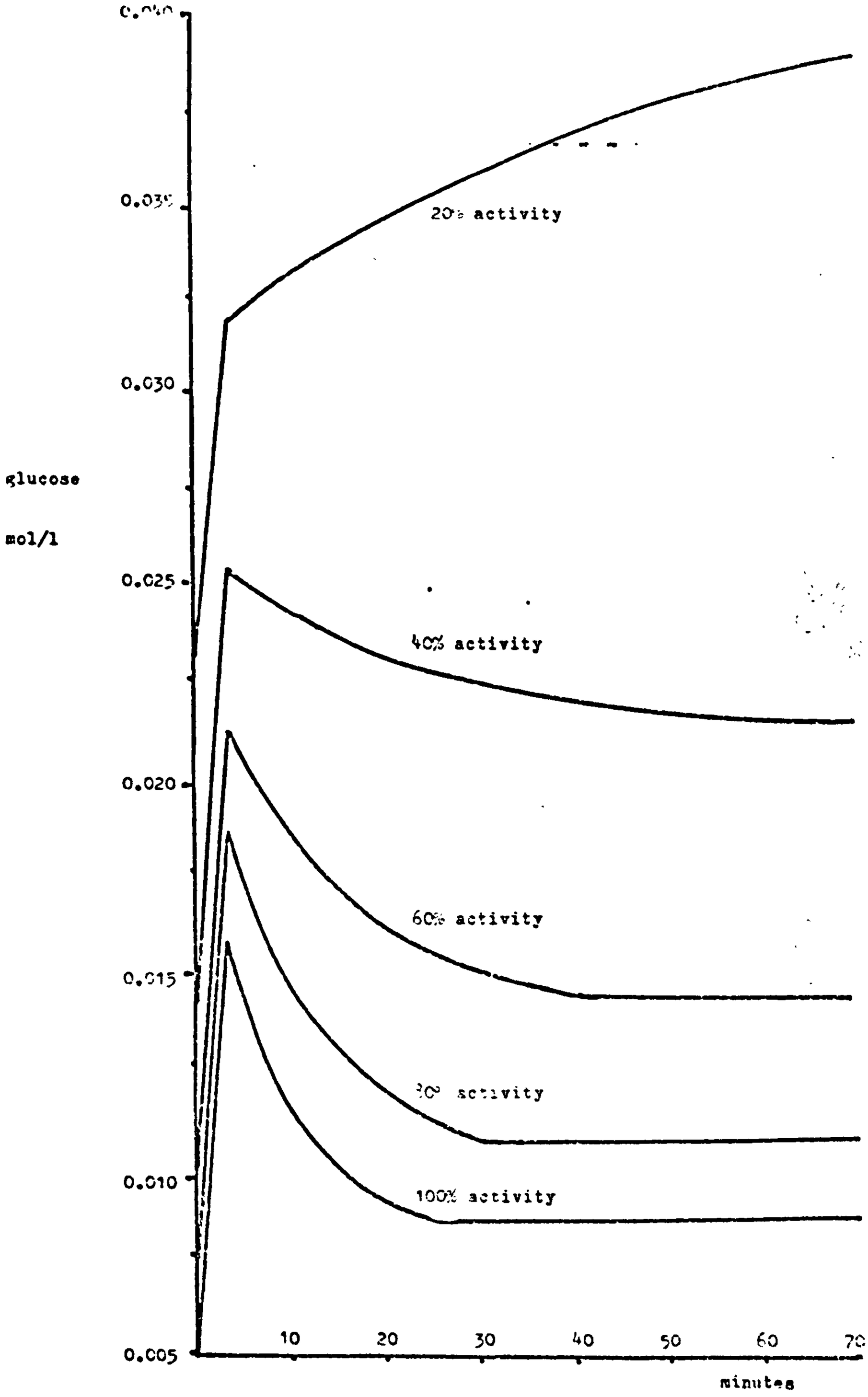


Figure 55 Model response to glucose infusion but with progressive reduction of hepatic glucokinase activity.

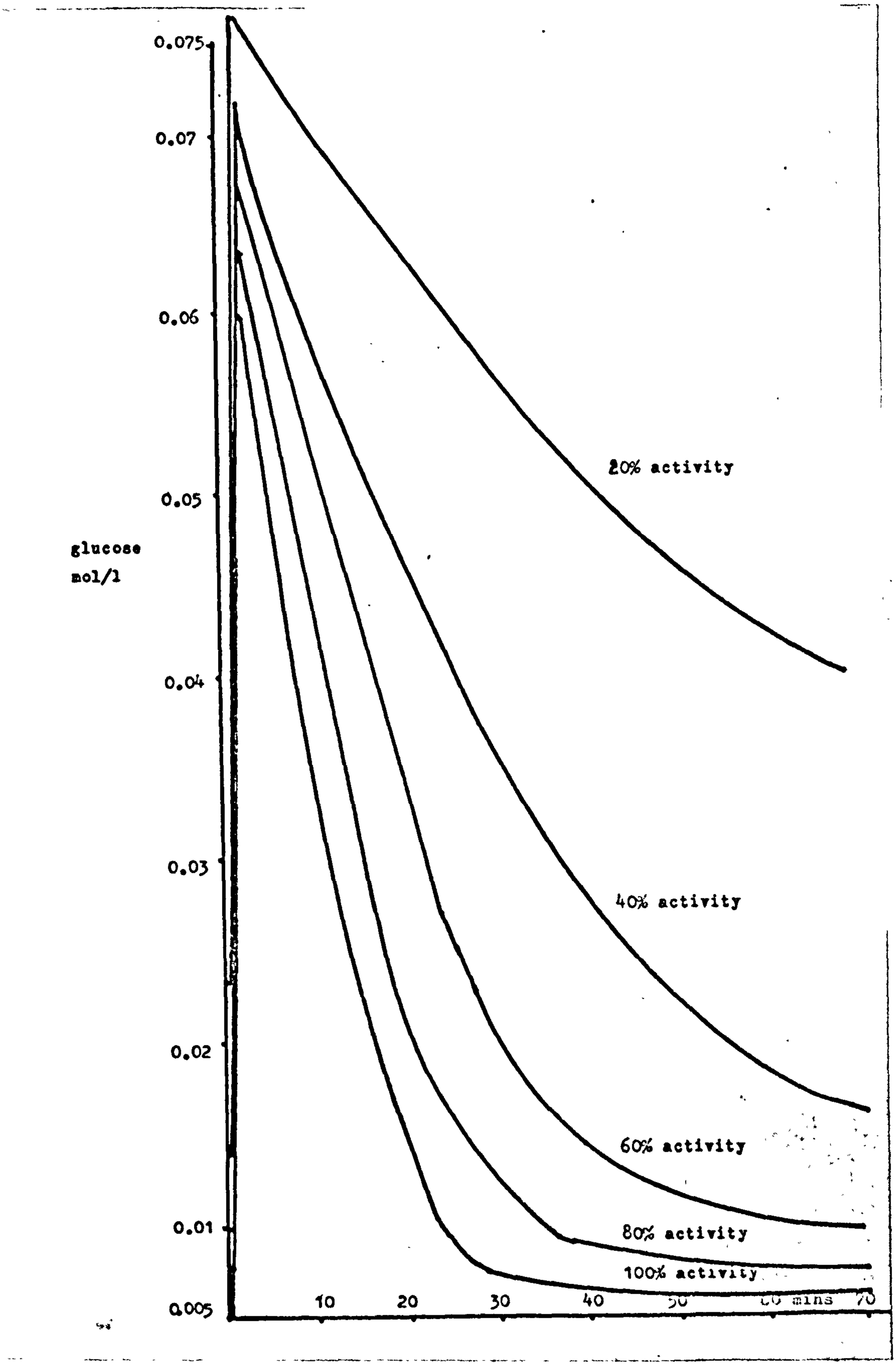


Figure 56 Model response to glucose injection but with progressive reduction of hepatic glucokinase activity.

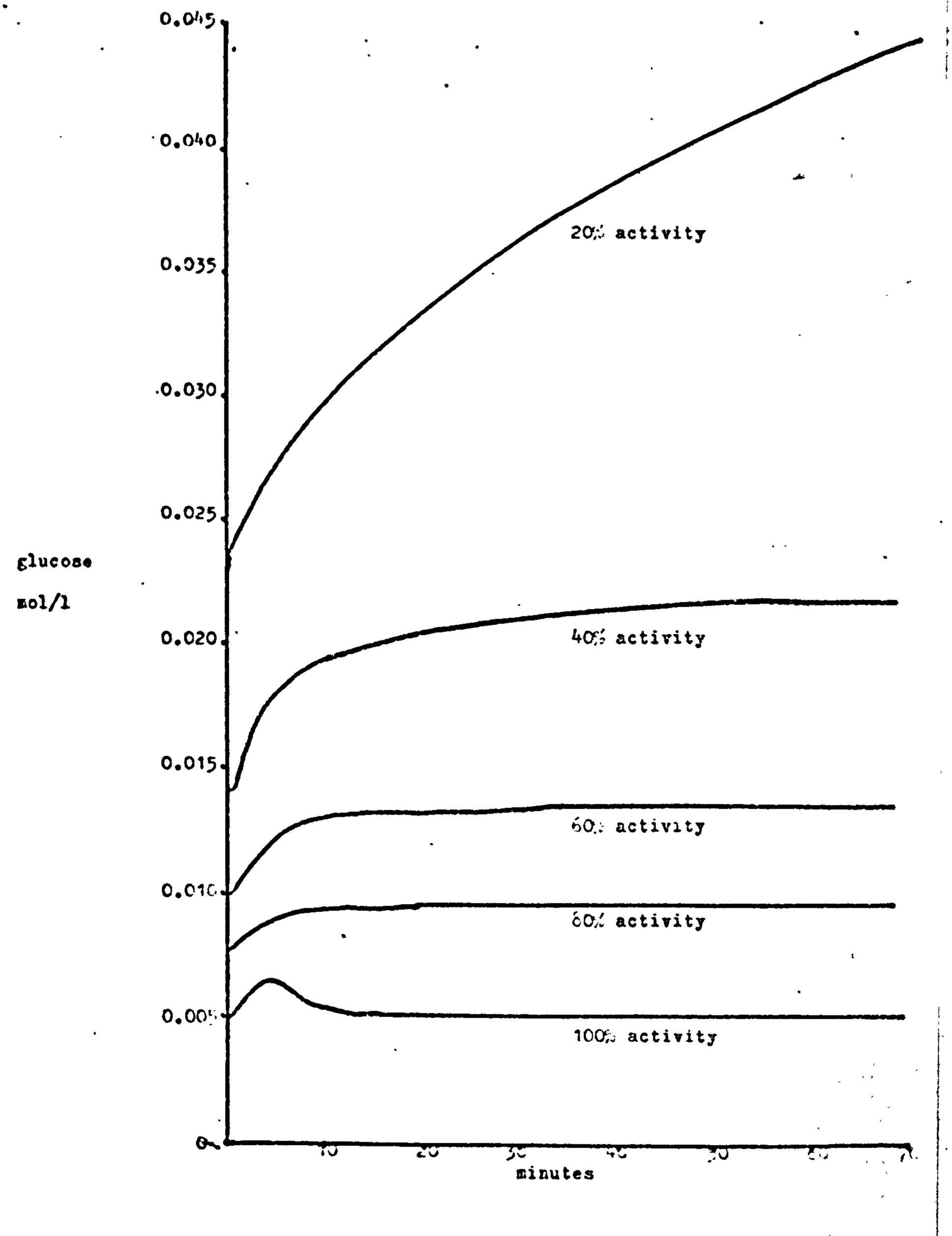


Figure 57 Model response to glucagon injection but with progressive reduction of hepatic glucokinase activity.

CHAPTER 8
DISCUSSION

In most models of glucose metabolism emphasis has been upon the development of block diagrams, deriving the transfer functions, or other mathematical description, for the blocks in the diagram, and then examining the closed loop response to various test inputs. These responses were then compared with responses in the real system. If the correspondence was not adequate mathematical manipulation of the model was often resorted to in order to obtain good fit. This all too often meant a sacrifice of physiological realism for a dubious statistically valid model.

The present model seems to offer several advantages over this type of formulation. First, unlike many of the previous models in which there was an implicit relationship with the physiology, the present model is explicitly based on known physiology and biochemistry, especially at the enzyme level. Because there is this degree of parallelism between the model and the real system (i.e. isomorphism) the model can be used for testing hypotheses concerning the system structure and parameter values and pathological states. Furthermore, such a model is of value in studying the control mechanisms involved at molecular and organ level in some detail.

Simulation results in response to the 4 test stimuli were presented in the last chapter and it can be seen that good general conformational agreement with experimental test data is achieved. There has been no attempt to quantify in statistical terms the differences between experimental and model responses. It was considered more important to obtain a model structure and parameter values that were physiologically valid.

As short term control was being studied primarily the model tends to focus on the role of the liver and the dominant enzyme systems. At an early stage an attempt was made to incorporate parts of the glycolytic sequence

but computer simulation suggested that their effect upon the system was not important. It seemed that the relationships of the glucose - glucose-6-phosphate - glycogen triangle were dominant. Other features included were insulin-glucagon control of glycogen production and glycogen dismutation, and the peripheral uptake of glucose.

Comment on results

Variation of hepatic hexokinase activity

The activity of hepatic hexokinase was progressively reduced. This was effected by changing the rate at which hepatic glucose-6-phosphate was produced for a given glucose concentration. This would be analogous to the situation thought to obtain in diabetes mellitus when there is an absolute insulin deficiency and consequently a reduction in the activity of the glucokinase component of the hepatic hexokinases.

This variation in glucokinase activity leads primarily to a change in the fasting plasma glucose steady-state concentration. For instance a reduction of 40% in glucokinase activity leads to a new glucose steady-state of 0.014M (= 252 mg/100 ml). There are corresponding and proportional changes in the circulating concentrations of insulin and glucagon.

In response to the intravenous injection of insulin the oscillations observed in the glucagon response under normal conditions are gradually removed as the activity of glucokinase progressively decreases.

Following a primed infusion of glucose when the glucokinase activity is very low a biphasin insulin response is obtained (~~Figure~~). The phases of this response correspond to the absolute elevation and the rate of change of glucose concentration of the priming dose and the constant infusion respectively.

This observation must call into question the views by various authors (e.g. Grodsky et al. 1972) concerning the physiological mechanisms involved in the pancreatic response leading to biphasic and multiphasic insulin

responses. It would appear that such responses could simply reflect the pattern of plasma glucose elevation and the rate of change of glucose concentration to which the pancreatic β-cells are responding. These patterns may alter as a result of differing physiological and pathological situations. These observations lead to the hypothesis that the derangements of pancreatic metabolism are secondary to extra-pancreatic changes, rather than the reverse. If this be the case the pancreatic response is correctly reflected by the insulin controller equation. The model also confirms that variation of glucokinase activity in the liver contributes significantly to the development of impaired glucose tolerance and to the rise in the fasting plasma glucose concentration as in diabetes.

The activity at the 20% level would be approximately that contributed by the haptic glucokinase which is the carbohydrate and insulin independent hexokinase. But even at the 80% level of activity (that is 20% diminution of activity) the response to injection of glucagon intravenously is abnormal. Instead of a rapid transient hyperglycaemic response, with reattainment of the fasting concentration within about 20 minutes, there is a continued elevation of glucose (~~see Figure~~). This switching of glucose to a new steady-state value is the result of glucagon being elevated above its threshold value thereby effectively preventing glycogenesis, partly by exhibiting the UDP-glucosyl transferase reaction and partly by accelerating the phosphorylase reaction. A similar effect can be obtained by altering the gain in the mathematical term relating to loss of glucose to the periphery or by varying the glucagon threshold level. As a result of this glucose switching the levels of insulin and glucagon are also switched.

The injection of glucose when hepatic hexokinase activity is impaired leads to a reduced rate of fall of glucose over the time course of the experiment.

Variation of peripheral hexokinase activity

Alteration of peripheral hexokinase activity leads to results rather similar to those discussed in the last section though to a markedly lesser degree. For instance, a 60% reduction in hexokinase activity in peripheral tissues brings about a new glucose steady-state concentration of 0.0089M (160 mg/100 ml).

There is a similar lack of glucagon oscillation following the insulin sensitivity test and again, glucose switching follows glucagon injection. However, this occurs when hexokinase is only slightly impaired. When hexokinase activity is down to as little as 40% of normal there is little change in the plasma glucose concentration, although there is a slight but perceptible shift.

The observations for both insulin and glucagon suggest that they both work through threshold mechanisms. The oscillatory form of the glucose response in all runs is also interesting, its association with glucagon suggesting the importance of the phosphorylase mechanism in this process. In this context it is also interesting that significant variations of plasma glucose levels are seen in the early stages of diabetes mellitus and also in obesity. There are situations when insulin 'resistance' may enhance glucagon 'sensitivity'.

The phosphorylase reaction

The phosphorylase reaction was deleted from the model with rather interesting results. The effect of removing this reaction would be to prevent mobilisation of hepatic glycogen, a situation analogous to a glycogen storage disease (Type 6 glycogenosis). There were slight changes in the steady-state levels in response to the test stimuli. The new steady-state glucose concentration achieved with the primed glucose infusion was reduced to 0.007M (138 mg/100 ml) compared with 0.0084M (151 mg/100 ml) in the control situation. Similar changes were observed following intravenous glucose

with corresponding changes in hormone steady-state concentrations. There was no fall of the fasting glucose concentration in either of these situations, and the conformation of the glucose injection and infusion curves appear to be normal. Furthermore, the injection of insulin did not yield the prolonged hypoglycaemia expected. This suggests that there is another mechanism in the gluconeogentic pathway, not identified for the purpose of the present model, whose activity must vary with, or fall in parallel, with the activity of phosphorylase. This is borne out by the fact that the fasting steady-state is regained so rapidly after insulin, which can only be due to the direct contribution of the gluconeogentic pathway.

Because, apart from minor switching effects, the dynamics of glucose are little altered by phosphorylase deletion, it must be concluded that the role of gluconeogenesis is concise in the maintenance of plasma glucose homeostasis, and that probably glycogen has only a poorly adapted buffer function, and further that glucose uptake is secondary in importance to gluconeogenesis in controlling the synthesis of glycogen.

The glucose-6-phosphatase reaction

The activity of the glucose-6-phosphatase reaction was altered by reducing the conversion rate of glucose-6-phosphate to glucose to 30% of its normal value. As was expected the fasting glucose concentration fell to within the hypoglycaemic range 0.0018M (34 mg/100ml); insulin levels were basal.

Following the primed infusion of glucose a steady-state was attained, after undershooting, 0.0026M (46 mg/100ml). In response to the injection of glucose there was a switching of the glucose to a steady-state value of 0.0013 M (25 mg/100 ml) with a multiphasic adrenaline response. However, despite glucose being switched to a lower level value insulin is raised to a level corresponding to its threshold. These observations seem to be of concise importance in that they explicitly confirm the role of the liver in

regaining the steady-state following perturbation (Soskin and Levine 1938; Cramp 1970). Furthermore, they provide an insight to the possible mechanisms of functional hyperglycaemia in which low glucose concentrations together with normal insulin levels are observed following a meal or oral glucose loading (Marks and Rose 1965).

After insulin injection the plasma glucose concentration remained depressed at about 0.0013M (25 mg/100 ml). Furthermore, the glucagon injection caused glucose to be transiently depressed before restoration of the steady-state at 0.0019. The latter observation is difficult to explain in physiological terms, but both results do highlight the two glucose states in which the system is able to switch from one to another under the influence of a pulse input.

Control of the system by insulin

Apart from the early phase immediately following a stimulus it would appear that it is the response to glucose elevation that dominates the situation. A change in the sensitivity to glucose elevation alters the insulin steady state; a threefold increase in sensitivity raises the concentration from 122 mU to 142 mU . Following glucose and glucagon stimuli a switching of steady-states is observed. The response to insulin itself is unchanged but for changes in the insulin steady-state value. These results suggest that alteration in the hormone sensitivity element (that is fine control) have little effect upon the metabolic processes involved. Threshold switching mechanisms appear to dominate for irrespective of changes in sensitivity to glucose changes the general confirmation of glucose-insulin relationships are unaltered.

Control of the system by glucagon

Varying the sensitivity of the glucagon controller to glucose elevation

gives rise to a variety of steady-state switching effects in response to the test inputs. However, unlike with insulin, not only the hormonal controllers but also the glucose steady-state can be switched. A threefold reduction in the glucagon sensitivity leads to a steady-state of 0.007M (126 mg/100 ml) following primed glucose infusion, whereas it is 0.00517 (90 mg/100 ml) following the intravenous injection of glucose. In both situations there are multiphasic adrenaline responses.

However, following variation of the glucagon threshold level there is an alteration of the system steady-state. Reducing the threshold level from 0.39 to 0.30 ug elevates the glucose steady-state to 0.006M (112 mg/100 ml).

Changes in the glucagon threshold level appear to bring about changes in steady-state values following perturbation of the system by altering the sensitivity of the glycogen - glucose-6-phosphate - glucose reaction sequence.

Some aspects of control

The results obtained from the model show that this study has produced a method for examining closely the variety of control mechanisms that are operating upon the metabolism pathways of glucose utilisation.

It would appear that during the short time scale of the simulations coarse control of the system is provided by hormones which are involved in threshold effects. Under these circumstances the hormones provide an environment in which providing the switching conditions are satisfied the appropriate reaction sequences are able to proceed. This would seem to be a particularly appropriate situation for use of the term 'permissive'. Furthermore, varying the sensitivity of the various hormonal controllers to glucose, whilst causing some steady-state changes, does not bring about significant changes in the conformational relationships.

The exception to these latter considerations would be the action of insulin in the periphery at very high concentrations, when a multiplier

effect provides an example for direct involvement of the hormone in peripheral glucose transport. This may not be surprising as insulin in the peripheral tissues is primarily involved in an active membrane transport mechanism. The evidence from this study does suggest however that hormones provide principally a coarse control mode for glucose metabolism.

The interactions of insulin and glucagon in an antagonistic manner provides an interesting example of what control engineers would call a two channel permissive/inhibitory control. However, what is interesting from a phylogenetic point of view is the number of hormones that could replace glucagon in this two-channel system. Notwithstanding that most of these hormones exhibit a variety of metabolic effects the fact that they all antagonise insulin action provide a puzzle in redundancy.

Fine control of glucose appears to reside in small changes of enzyme controlled biochemical dynamics. Glucokinase and hexokinase are both examples of enzymes in which comparatively small changes in enzyme activity can produce substantial changes in the steady-state concentration of glucose.

Several examples of steady-state switching have been cited that, although biochemical phenomena, could be analysed in terms of finite state machine behaviour. Indeed, the application of automata theory to biochemical problems has received particular attention from Higgins (1963), and it would seem that further development is required.

Application of control theory

However, the application of the techniques of control theory to metabolic problems at the bipchemical level is bedevilled with problems. There are probably two important reasons for this. Firstly, we are concerned with the control properties of very complex systems; complex in the sense that there are involved numerous different component sub-systems interacting in a variety of ways. Secondly, there is no established general theory of control of

biochemical systems. As succintly stated by Kacser and Burns (1973) - "there are bits of theory, but no comprehensive theory."

One 'bit' of theory is represented by maps of metabolic pathways that give information on the structure of the system: that is information about biochemical transformations, syntheses and degradations. This 'molecular anatomy' says 'what goes' but not 'how much'. This area, another 'bit' of theory, is covered by enzymology, with its data on individual enzymes, their kinetic inhibition and activation characteristics, molecular structure and as yet some rudimentary ideas about the theory of catalysis.

Such discussions that there have been of 'control' and 'regulation' in biochemical systems (see for example Newsholme and Start 1973) tend to centre on the question of which enzymes are controlling the flux in a pathway metabolic and their experimental identification, invariably <u>in vitro</u>. This identification involves measurement of enzyme kinetics and pool sizes, under various conditions. Such discussions suggest that controlling enzymes can be identified as those that satisfy a number of different criteria (see e.g., Newsholme and Gevers, 1967; Scrutton and Utter 1968; Newsholme and Start 1973) which are necessarily related to the measurements. There does seem to be in this approach a lack of definition of the various aspects of control to which these criteria are applicable. The establishment of necessary and appropriate definitions and their relationships needs a rigorous theoretical approach.

This thesis describes an attempt to provide such an approach, albeit elementary, that is based on functional information available in the literature. The purpose of the work was to obtain some insight into control at the sub-system level in man which would provide new ideas for experiment, diagnosis and therapy.

The model does yield information which, for critical reasons, it would be difficult to confirm in man, but does corroborate observations made in the experimental animal. The plasma glucose dynamics certainly reflect in general

terms those observed in man following perturbation. It may therefore be confidently concluded that the parallelism of the model with the 'real' physiological system is good inasmuch as the important control elements have been reproduced in the model. It would also appear that assumptions concerning the use of kinetic data observed *in vitro* in predicting the physiological characteristics of this particular system *in vivo* are valid.

Of course, as is true for all theoretical formulations, the present model, though based on sound assumptions concerning its biochemical and physiological structures, may not be the unique model of plasma glucose dynamics even though it predicts many recognisable physiological characteristics. Nevertheless, it has highlighted the possibility that fine control of plasma glucose resides intrinsically within individual enzyme reaction systems rather than being dependent solely on circulating hormone levels. This provides an avenue that deserves further investigation.

Conclusions

A mathematical model has been described that emphasises the importance of the fundamental structure of the system and multivariate control. It is soundly and explicitly based upon current biochemical and physiological concepts. This makes possible the investigation of both normal and abnormal glucose metabolism and their control processes. The physiologist is provided with a tool for testing hypotheses concerning metabolic systems behaviour, and obtaining insight into the functioning of the system at various levels.

Two levels of short term control have been identified within the glucose system; hormonal elements appear to provide a coarse control whilst enzymes appear to provide the fine control and autoregulatory effects. It does appear that hormones primarily maintain the biochemical environment of the organism whilst the control of flux within metabolic sequences is intrinsic to certain enzymes within the system.

APPENDIX I

THE MICHAELIS-MENTEN KINETIC MODEL AND THE BRIGGS-HALDANE MODIFICATION

The mathematical basis for the kinetics of the fundamental catalytic process of enzymology was established in the classical paper of Michaelis and Menten (1913). Their starting point was the following stepwise mechanism for explaining the conversion of a substrate S to product P by the enzyme E :

$$E + S \xrightarrow{k_1} ES \qquad \text{(Association) (Rapid) ( )} \qquad \text{A.1.1}$$

$$ES + \xrightarrow{k_2} E + S \qquad \text{(Dissociation) (Rapid) ( )} \qquad \text{A.1.2}$$

$$ES \xrightarrow{k_3} E + P \qquad \text{(Decomposition)(Slow) ( )} \qquad \text{A.1.3}$$

Where k_1, k_2 and k_3 are the deterministic rate constants of the corresponding reaction steps. Thus it was postulated that a molecule of enzyme, unlike an inorganic catalyst, actually enters into the reaction process forming an 'enzyme-substrate complex' molecule ES . The ES molecules are dissociable so that an equilibrium is attained rapidly between the reactants E, S and the intermediate species ES . Michaelis and Menten postulated that this equilibrium state was not disturbed by occasional decompositions of the ES molecules that give rise to the product P and the liberated enzyme molecule. The latter is free to complex other substrate molecules sequentially; thus is derived the concept of the "turnover number" of an enzyme (usually of the order 10^5 mol $cm^{3^{-1}}$ sec^{-1}). Reactions of this type have a low enzyme requirement in relation to substrate concentration.

This hypothetical mechanism led to the classical hyperbolic Michaelis-Menten equation relating v , the instantaneous rate of appearance of products, with [S] , the concentration of substrate present at time t after the start of the reaction :

$$v = \frac{V[S]}{\frac{k_2}{k_1} + [S]} \qquad \text{Michaelis-Menten equation (equilibrium theory)} \qquad \text{A1.4}$$

Where V is the maximal value of v, attainable when the total enzyme E_T is complexed so that $V = k_3 E_T$.

This concept of the "equilibrium assumption", that is, the requirement that the rate of decomposition of the complex ES into products be slow enough so as not to disturb the continuously maintained association-dissociation equilibrium was considered by several workers to be an over-simplification. They claimed that the fullest solution could only be obtained by solving the complete system of differential equations comprising the mathematical model, that is, the differential equation system based on the mechanism of Equations A1.1, A1.2 and A1.3.

The two independent equations that form the canonical system are :

$$\frac{d(ES)}{dt} = -(K_2 + K_3)[ES] + K_1 E_T [S] - K_1 [ES][S] \qquad \text{A1.5}$$

$$\frac{d[S]}{dt} = K_2 [ES] - K_1 E_T [S] + K_1 [ES][S] \qquad \text{A1.6}$$

and where $E_T = E + ES$.

This system has not been solved completely but a series solution is obtainable both for ordinary points and in the neighbourhood of singular points (Bartholomay, 1960).

Briggs and Haldane (1925) replaced the equilibrium assumption of Michaelis and Menten with the pseudo-steady-state assumption $d(ES)/dt = 0$. Using this with equation A1.5 leads to

$$v = \frac{V[S]}{Km + S} \qquad \text{Briggs-Haldane equation (steady-state theory)} \qquad \text{A1.7}$$

This has the same form as the Michaelis-Menten equation but with the generalised Michaelis-Menten constant $Km = (K_2 + K_3)/K_1$ replacing the term K_2/K_1 in the denominator of equation A1.4.

APPENDIX II

THE CALCULATION OF KINETIC CONSTANTS FOR THE PHOSPHORYLASE REACTION

The rate equation

$$v = \frac{(V_1 \cdot K_{G1P} \cdot P) - (V_2 \cdot K_p \cdot G1P)}{(K_p \cdot G1P) + (K_{G1P} \cdot P) + (K_p \cdot G1P)\left(\frac{K_A}{AMP}\left(\frac{G6P}{K_{G6P}} + 1\right) + 1\right)} \quad (A2.1)$$

was derived using the enzymatic mechanism cited already in Table II K_p and K_{G1P} are the respective kinetic constants for P_1 and glucose-1-phosphate; K_A and K_{G6P} respective dissociation constants for AMP and glucose-6-phosphate. All these constants are independent of substrate, activator or inhibitor concentrations.

In the absence of glucose-6-phosphate and the substrate, glucose-1-phosphate say, the above equation reduces to

$$v = \frac{V_1 \cdot P}{P + K_p'} \quad (A2.2)$$

with $K_p' = K_p\ (K_{AMP}/AMP + 1)$. This latter expression implies that the observed kinetic constant for P_i, K_p', decreases with the activator AMP, but that the plot of K_p' against 1/AMP has a slope K_p, K_{AMP} and intercept K_p. K_p and K_{AMP} are found by this method using the data of Helmreich and Cori (1964). Equation (A2.2) can further be rearranged

$$= \frac{V_1 \cdot AMP}{AMP\ \frac{P + K_p}{P} + K_{AMP}'} \quad (A2.3)$$

in which $K_{AMP}' = (1/P)\ (K_p)\ K_{AMP}$. This expression implies that K_{AMP}' the observed kinetic constant for AMP decreases with P_i or glucose-1-phosphate,

and that a plot of K'_{AMP} against $1/P\ K_p.\ K_{AMP}$, thus leaving K_p far above K_{AMP} may be obtained. Using the equations (A2.2) and (A2.3) and the Helmreich and Cori (1964) data respective values of K_{AMP} of 3.2×10^4M and 3.6×10^4M are obtained.

If glucose-1-phosphate is the substrate instead of phosphate equation (A2.3) yields $K'_{AMP} = (K_{G1P}/G1P)\ K_{AMP}$ from which K_{G1P} can be calculated from measurements of K'_{AMP} at different glucose-1-phosphate concentrations if K_{AMP} is known.

In the absence of inorganic phosphate equation (A2.1) rearranges to give

$$= \frac{\dfrac{V_2.\ AMP}{(G1P + K_{G1P})}\ AMP + K''_{AMP}}{G1P} \qquad \text{(A2.4)}$$

where $$\frac{K''_{AMP}}{G1P'} = \frac{K_{G1P}\ K_{AMP}}{G1P'}\left(\frac{G6P}{K_{G6P}} + 1\right)$$

K_{G6P} can be determined from measurements of K''_{AMP} made at constant glucose-1-phosphate and AMP concentrations because K_{G1P} and K_{AMP} are known.

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ADDENDUM

In order to clarify certain passages, expand on others, and include textual corrections, this addendum is submitted to supplement the thesis "The Modelling of Blood Glucose Dynamics in Man" examined on 11 July 1975.

D. J. Cramp.

8. viii. 75.

CORRECTIONS

P.16	line 21	Insert 'that' to read 'a view that was ...'
P.18	line 5	Delete 'estimates' insert 'estimations'
P.32	line 21 last word	Spelling of 'phlebitis'
P.34	line 17	Insert 'and' to read '(EC 3.1.3.9) and of'
P.41	line 25	Delete 'with' insert 'will' Spelling of 'concomitantly'
P.50	last line	Should read 'equations (1) to (4)'
P.65	line 1	Read 'injections' not 'injection'
P.70	last line para. 2	Delete 'epinephrine' insert 'adrenaline'
P.84	Section II (ii)	Delete 'and adrenaline'
P.89	last line	Should read '3.2 1' <u>not</u> '3.21'
P.93	under heading	'Intravenous glucose injection' insert 'litre^{-1}' to read '0.0304 mol min^{-1} litre^{-1} '
P.94	at top of page	Insert 'litre^{-1}' to read '0.000434 mol min^{-1} litre^{-1}'
P.97	para. 2 line 1 line 2	 Should read 'series of test impulses;' Spelling of 'intravenous'
P.149	para. 2 line 3 last line	 Delete 'concise' insert 'critical' Delete 'concise'
P.152	line 12	Should read 'provides a puzzle'
P.153	line 6 last para. line 1	Insert 'latter' to read 'This latter area ...' Delete 'critical' insert 'ethical'

Page 43 et. seq. Development of section on gluconeogenesis

The path of carbon in glycolysis and gluconeogenesis is depicted in Figure S.1. The two pathways of glycolysis and gluconeogenesis are closely related and as gluconeogenesis is opposed by glycolysis it is necessary to have mechanisms able to alter the relative rates of these two opposing pathways in order to provide predominance of one or the other. Consequently, the regulatory processes must be concerned with two important aspects of control, namely substrate concentration and direction of metabolic flux.

Krebs (1957) suggested that, possessing detailed knowledge of a metabolic pathway, it is possible to predict which enzymes are advantageous for metabolic control, and he went on to point out possible sites of control in a pathway, namely:

1. The initial enzymatic step.
2. Substrate transport.
3. Enzymes controlling reactions immediately after a branch point.
4. Enzymes catalysing two separate reactions comprising the forward and back reaction of one step.

Thus Newsholme and Gevers (1967) point out that regulatory steps in these pathways will be probably found at the following sites:

1. Membrane transport of the major substrates, namely amino acids, pyruvate, lactate, fructose, and glycerol.
2. Principal metabolic branch points relating to disposal of pyruvate, oxaloacetate, phosphoenolpyruvate, triose phosphates, and glucose-6-phosphate.

3. Enzymes specific for either pathway at undirectional sites, namely hexokinase, phosphofructokinase, and pyruvate kinase for glycolysis, and pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-diphosphatase, and glucose-6-phosphatase for gluconeogenesis.

4. Reactions where nucleotides are cosubstrate such as the kinases and glyceraldehyde-3-phosphate dehydrogenase.

The most likely candidates for involvement are pyruvate carboxylase and pyruvate dehydrogenase; phosphoenolpyruvate carboxykinase (involving malate dehydrogenase, citrate synthase and aspartate aminotransferase); pyruvate kinase and glyceraldehyde-3-phosphate dehydrogenase; fructose diphosphatase; phosphofructokinase, glucose-6-phosphatase and hexokinase.

It is in hepatic tissue that the interrelationship between the glycolytic and gluconeogenetic pathways appear to be most closely subjected to control.

Control of gluconeogenesis by substrate availability

The main precursors for glucose formation in the liver are glycerol, amino acids, and lactate from glycolysis in extrahepatic tissues (Figure S.1.). Experiments with the perfused rat liver suggest that an increase in the plasma concentration of any of these precursors could result in a stimulation of gluconeogenesis, (for reviews see Exton, 1972; Exton et al., 1973). For example, the half-maximal rate of gluconeogenesis from glycerol is obtained at a concentration of 0.5 mM: the plasma level of glycerol in the fed animal is 0.1 mM and this is doubled during starvation. However,

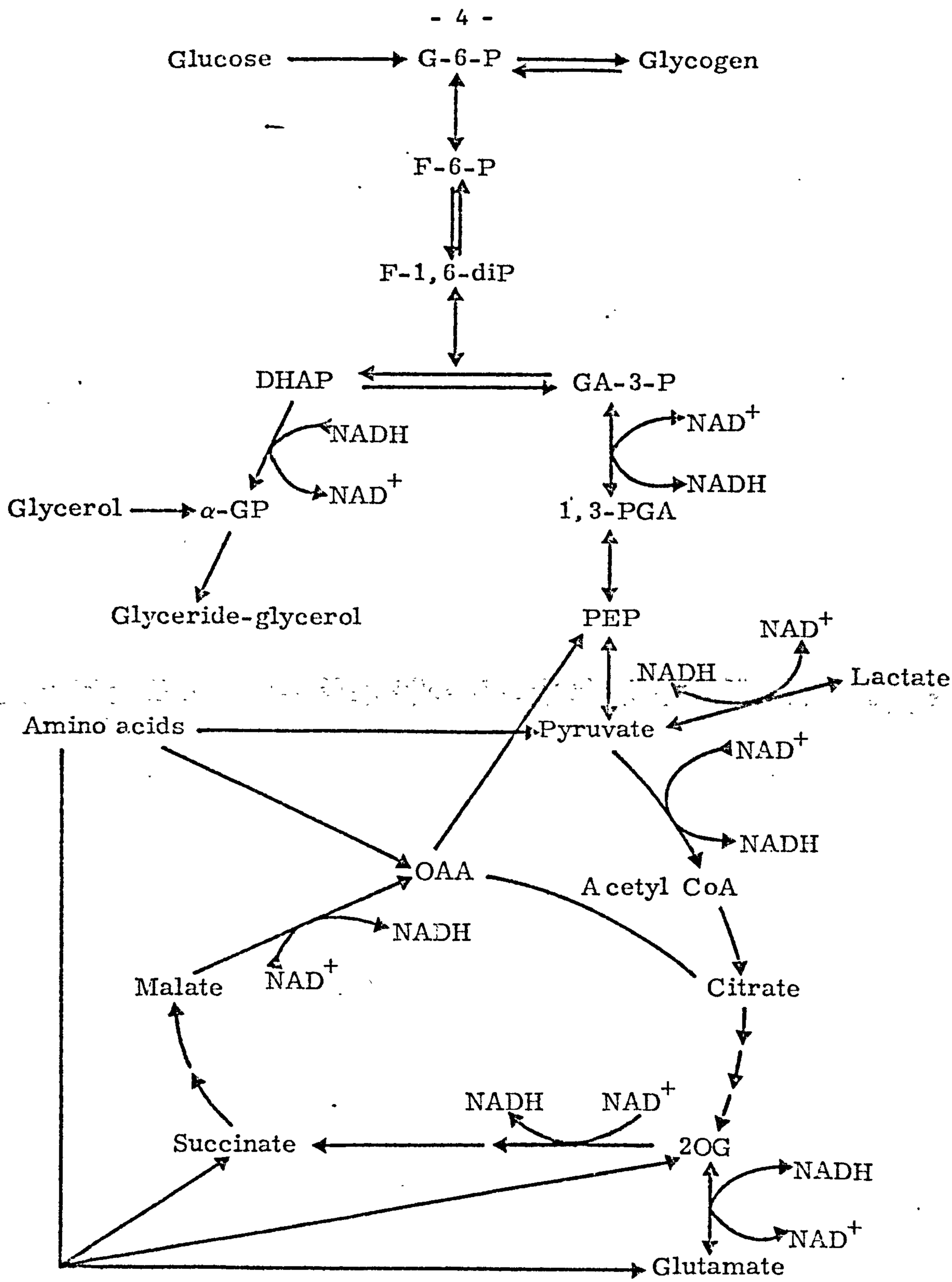

Figure S.1 The glycolytic and gluconeogenetic pathways of the liver

increase substrate availability for gluconeogenesis could also arise from a stimulation of amino acid transport into the liver cell.

There is some evidence that this transport process is modified by hormones: for example, glucagon stimulates the transport of alanine into the liver cell. It is not known if this hormone stimulates the transport of all the amino acids but the inward transport of lysine and of the non-metabolisable amino acids, α-aminoisobutyrate and cycloleucine, are also increased.

Amino acids which function as precursors for gluconeogenesis are obtained from the breakdown of protein in muscle. This catabolism is increased due to changes in the circulating concentration of glucocorticoids. Lack of insulin will result in a depression of protein synthesis, whereas the glucocorticoids stimulate protein degradation. The biochemical mechanisms by which proteins are degraded are largely unknown and therefore it is not possible to propose a plausible theory for the action of glucocorticoids. However, the subject of control of protein concentration by effects on synthesis and degradation has been well reviewed by Schimke (1969).

The plasma lactate concentration can increase to very high values (20 mM) during vigorous exercise and in lactic acidosis. This would undoubtedly stimulate gluconeogenesis, and the liver and kidney cortex play an extremely important role in reducing the concentration of plasma lactate. However, it seems unlikely that an increase in the concentration of lactate alone can lead to increased activities of all the gluconeogenic enzymes. Therefore the possibility that lactate could produce changes within the liver that could stimulate gluconeogenesis has been investigated. Administration of lactate to an intact animal raises the content of pyruvate in the

liver and this should stimulate pyruvate carboxylase. The administration of lactate also increases the hepatic content of citrate and this would inhibit PFK and hence lead to stimulation of fructose diphosphate hydrolysis (Newsholme and Start, 1973).

Regulation of gluconeogenesis by hormones

Of recent years a great deal of experimental work has been carried out to investigate the effects of hormones on gluconeogenesis both in the intact animal and in the isolated perfused liver. Unfortunately, it is still not possible to formulate a satisfactory theory of control of the gluconeogenic enzymes by hormones. The effects of hormones on gluconeogenesis were reviewed by Newsholme and Gevers (1967), since when the major developments in this field stem from the work of Exton and Park who have used the isolated perfused liver to study the stimulation of gluconeogenesis by glucagon, adrenaline, and glucocorticoids and its inhibition by insulin (Exton, 1972; Exton et al., 1973).

(a) Effect of glucagon on gluconeogenesis

Newsholme and Gevers (1967) suggested that the effects of glucagon on gluconeogenesis might be explained by a stimulation of hepatic lipolysis and that the resulting increase in fatty acid oxidation would lead to elevated concentrations of acetyl-CoA and citrate. Gluconeogenesis would be stimulated as described above. However, in careful, precise experiments with the perfused liver, Exton and co-workers have shown that the effect of glucagon on gluconeogenesis occurs at physiological concentrations of the hormone, whereas the effects on lipolysis and ketosis occur at concentrations that are higher than physiological. These workers have also shown that only very high concentrations of fatty acids are effective in the stimulation of gluconeogenesis. Even at a concentration of 1.8 mM,

fatty acids (bound to a physiological concentration of albumin) did not cause a stimulation of gluconeogenesis although the rate of ketone body formation was increased and thus presumably the content of acetyl-CoA was also increased (although it was not measured). Exton and Park conclude that the acetyl-CoA effect on pyruvate carboxylase is unimportant in the control of gluconeogenesis and that glucagon must stimulate gluconeogenesis by other means.

(b) Cyclic AMP and gluconeogenesis

Both glucagon and adrenaline stimulate adenylcyclase in liver and increase the content of cyclic AMP. Therefore, the stimulatory effect of these hormones upon gluconeogenesis may be exerted through an increase in the concentration of cyclic AMP. The evidence in support of this is: the effect of glucagon upon gluconeogenesis is very rapid; stimulation of ^{14}C incorporation from [^{14}C] lactate into glucose being detectable within 40 seconds of addition of glucagon to the perfused liver. The concentration of glucagon that stimulates gluconeogenesis half-maximally is the same as that which stimulates glyconeogenesis half-maximally and is the same as that which stimulates glycogenolysis and adenylcylase (i.e. 2×10^{-10} M) (Exton et al., 1972). The addition of cyclic AMP to isolated rat liver perfusate stimulates gluconeogenesis (as measured by ^{14}C incorporation from [^{14}C] lactate into glucose) (Exton et al., 1973).

The effects of glucagon, cyclic AMP, and adrenaline on the contents of gluconeogenic intermediates in the perfused liver are very similar. In particular, a 'cross-over' is observed between pyruvate and phosphoenolpyruvate (i.e. pyruvate concentration is decreased and phosphoenolpyruvate concentration increased). Thus

the hormones (and therefore also cyclic AMP) increase the conversion of pyruvate to phosphoenolpyruvate. Exton et al. (1972) rule out the possibility that this is due to an inhibition of pyruvate kinase (and an increase in phosphoenolpyruvate formation due to a substrate cycle) by demonstrating that glucagon has no effect on the conversion of fructose or dihydroxyacetone to lactate. Therefore, it can be concluded that cyclic AMP increases the activity of either pyruvate carboxylase or phosphoenol pyruvate carboxykinase (or both). Further experiments involving the incorporation of ^{14}C from [^{14}C] pyruvate into malate and phosphoenolpyruvate have suggested that phosphoenol pyruvate carboxykinase is the enzyme whose activity is modified by glucagon (Exton et al., 1971). Unfortunately, there is virtually no evidence that cyclic AMP affects the activity or therproperties of the enzyme in vitro.

(c) Effect of insulin on gluconeogenesis

Addition of insulin to the perfused liver results in inhibition of gluconeogenesis (and glycogenolysis). Whereas, treatment of rats with insulin antiserum increases gluconeogenesis and glycogenolysis. The administration of insulin antiserum to rats increases the content of cyclic AMP in the perfused liver and this is counteracted by the addition of insulin to the perfusate. However, addition of insulin to the perfusate when there is no antiserum treatment leads to a small decrease in the content of cyclic AMP. Furthermore, insulin inhibits the stimulatory effect of glucagon on gluconeogenesis (and glycogenolysis) in the liver and it prevents the rise in cyclic AMP content that is observed in the presence of glucagon alone (Exton

et al., 1973). Hence the physiological effects of insulin on the liver, like those of adrenaline and glucagon, may involve changes in the concentration of cyclic AMP.

However, recently Matsuura, Cheng and Kalant (1975) have presented evidence that in the rabbit glucose-6-phosphatase is the control point for hepatic glucose production and that its activity is controlled by insulin which acts as an inhibitor. These observations were the opposite of those obtained in the rat in which insulin produced a transient rise in glucose-6-phosphatase activity. So it is probable that significant species variation occurs.

(d) Effect of glucagon; insulin ratios on hepatic gluconeogenesis

Even though insulin and glucagon have antagonistic effects on hepatic gluconeogenesis, it is still not clear whether or not the hormones are competitive antagonists. As mentioned above, many of the glucagon effects appear to be mediated by increasing hepatic concentration of cyclic-3',5'-AMP while insulin opposes this process by interfering with the action, production or degradation of the cyclic-3',5'-AMP.

In vivo insulin and glucagon are simultaneously conveyed to the liver from the pancreas via the portal vein, and it is probable that the glucagon:insulin ratio in the portal vein may be cardinal in controlling hepatic glucose metabolism. Unger (1971) reviewed the alterations of glucagon:insulin ratio in various physiological and pathological states and suggested that the ratio may be of importance in determining the catabolic or anabolic state of the liver. However, in a recent paper (Parrilla, Goodman and Toews,

1974), evidence was presented suggesting that the ratio affected essentially hepatic glycogenolysis and that gluconeogenesis was stimulated only when the glucagon:insulin ratio was probably unphysiologically high in the portal vein.

(e) Effect of glucocorticoids on gluconeogenesis

Glucocorticoids increase the rate of gluconeogenesis by a mechanism which is at present unclear. In the rat, adrenalectomy markedly decreases the rate of ^{14}C incorporation into glucose from [^{14}C] lactate in perfused livers from fasted or diabetic animals. However, there is no effect upon the perfused livers taken from fed animals. Administration of cortisol to diabetic animals before sacrifice improves the incorporation of ^{14}C into glucose in the perfused liver. Analysis of the intermediates of gluconeogenesis shows that adrenalectomy decreases the rate of pyruvate conversion to phosphoenolpyruvate (Exton et al., 1973). The mechanism is unknown but possibly the glucocorticoids play a 'permissive role', which somehow increases the response of the gluconeogenic pathway to cyclic AMP. Glucagon is ineffective in activating gluconeogenesis in liver from fasted, adrenalectomised rats, but the response to glucagon is restored if dexamethasone is added in vitro or in vivo. Since adrenalectomy does not interfere with the ability of glucagon to increase the content of cyclic AMP in the liver, corticosteroids may influence the reaction between cyclic AMP and the gluconeogenic enzyme whose activity is modified by glucagon. The precise identification of this enzyme and some knowledge of how it is modified by cyclic AMP will doubtless aid the understanding of the action of both glucagon and glucocorticoids on gluconeogenesis.

The influence of the adrenal cortex on carbohydrate metabolism has been well documented since Hartmann and Brownell (1934) demonstrated in the dog that the severity of pancreatic diabetes was ameliorated by total adrenalectomy while the hypoglycaemic response to insulin was increased. This association between adrenalectomy and hypoglycaemia had been recognised by Bierry and Malloizel in 1908, and was only confirmed by Long and Lukens (1936) although the latter paper is better known. The reverse effect, the production of steroid diabetes due to glucocorticoid excess is as well recognised (Ingle, 1941).

The metabolic effects of glucocorticoids are opposed by insulin (Ashmore, 1964) and in the regulation of carbohydrate, lipid, and protein metabolism, these hormones play a key role, for insulin increases peripheral glucose utilisation, inhibits peripheral lipid mobilisation and modifies extra-hepatic protein synthesis, whilst, in the liver, the hormonal roles are reversed; glucocorticoid stimulates the de novo enzyme and protein synthesis while insulin acts as an inhibitor. The interplay of insulin and corticosteroids in the regulation of hepatic enzyme synthesis is of great importance (Weber, Singhal and Srivastava, 1965).

The hepatic effects of the glucocorticoids involve concurrent action in peripheral tissues, for the steroids release gluconeogenic amino acids from muscle and glycerol and lactate from adipose tissue. These precursors are carried to the liver through the blood stream and by saturating the hepatic gluconeogenic enzyme systems, contribute towards the formation of new hexose molecules. Further, the steroids release non-esterified fatty acids (NEFA) which act as the signal molecules to the switching mechanism that brings about glucose

production. The intra-hepatic metabolism of NEFA also increases the acyl-CoA level which activates pyruvate carboxylase. Further, these free fatty acids provide the reducing equivalents which promote an increase in the level of NADH, which inhibits pyruvate kinase and phosphofructokinase. The increased level of gluconeogenic metabolites also stabilises the gluconeogenic enzymes by saturating and protecting the catalytic centre. These acute actions of the steroids are manifest in a few minutes.

The acceleration of gluconeogenesis by glucocorticoids in experimental animals was originally explained in terms of enzymatic induction. Several groups of workers, Ray et al. (1964), Greengard et al. (1963), Weber et al. (1964), have demonstrated that actinomycin D, an RNA synthesis inhibitor, suppresses completely the induced elevation of enzymatic activity, but carbohydrate synthesis is only partly suppressed. These findings that gluconeogenesis can be accelerated independently of de novo hepatic enzyme synthesis suggested that there is regulation at the level of substrate control, that is by substrate supply.

In rats, as shown by Smith and Long (1967) an increased supply of amino acids from the peripheral tissues occurs under the influence of corticosteroids, and these authors suggest that this is an important factor in the high rates of gluconeogenesis in severe diabetes mellitus. Also, Betheil et al. (1964) showed significant changes in the plasma of rats treated with steroids, namely an increase in glutamate, aspartate, and alanine, of 58%, 25%, and 6%, respectively.

Although as Krebs (1964) has demonstrated there are many steps in intrahepatic metabolism that may be rate-limiting, the key reactions that are influenced by the reductive environment of the cell are those that are NAD dependent (see Figure S.1.). Equilibration of lactate, glutamate and α-glycerophosphate with their oxidised partners are determined by the $NADH/NAD^+$ ratio prevailing, an increase in this ratio diminishes utilisation of these precursors for gluconeogenesis and the oxidised partners are removed for gluconeogenesis.

Continuous substrate oxidations that are necessary for gluconeogenesis could also significantly challenge the 2-oxoglutarate and pyruvate dehydrogenase steps by diminishing the availability of NAD^+, but what is as important is that the pyruvate dehydrogenase reaction is exquisitely sensitive to NADH inhibition causing a decrease in the oxidative decarboxylation rate of pyruvate which could be carboxylated via the pyruvate carboxylase reactions to oxaloacetate. Generation of oxaloacetate by this reaction is a major rate-limiting step in gluconeogenesis (Utter and Keech, 1963), this substrate oxidation could attenuate the activity of this enzyme by diminishing acetyl-CoA concentration, the allosteric activator, and at the same time allowing accumulation of pyruvate which would equilibrate with lactate.

P.76 Note on paragraph on "Enzyme Mechanisms"

Enzyme reaction rates have been specified in terms of results obtained from in vitro experiments described in the literature. Most of the work described has been performed using either the rat or the rabbit as the source of e experimental material. Whether results obtained from these sources can be directly extrapolated to the human situation may be open to some doubt. However, there is as yet minimal information available to allow detailed kinetic properties of the human enzymes to be included in the model, so animal results must be used.

P.93 Results for insulin and glucagon dynamics were calculated from values obtained from the papers of Turner et al., 1971; Genuth, 1972; Goodner and Porte, 1972; Unger, 1972.

Further Comments on Chapter 7: 'Results'

Note The glucose value on the ordinate of each graph represents glucose concentration in mol/l not mmol/l.

The graphs shown in the Results Section are those selected from approximately 3000 computer runs involving 29 model simulations (see pages 102-104). Each simulation model was derived from the original mathematical model by progressively varying parameter values in the model equations until an acceptable configuration of the glucose and hormone response curves was obtained. The simulation models were all subjected to three provocative test stimuli, representing the intravenous glucose tolerance test, a primed glucose infusion, and the intravenous injection of an insulin bolus. However, the model simulations numbered 19 to 29 were also subjected to the stimulus representing an intravenous glucagon injection.

Consideration of the figures in the Results Section

Figure 20 shows the result of a simulation without any input into the system in the form of a test stimulus. It can be seen that the model allows any 'false' transients to decay and 'true' steady state to be obtained.

Figures 21-26 represent the graphical plots of data obtained for the intravenous glucose injection. Those selected represent the results obtained by implementing the parameter conditions of simulation models 2, 6, 9, 11, 25, and 26. There does appear to be gradual improvement towards the configuration normally seen of glucose and hormone response curves with progress through models 2, 6, and 9, by

(a) altering threshold values for hepatic glucagon, which would affect hepatic glucose output, and (b) introducing a peripheral threshold effect for insulin and altering the sensitivity of the phosphorylase equation whereby the glucose-6-phosphate pool can be replenished in response to the new glucagon threshold. An even more acceptable curve configuration shown in Figure 25 was obtained from the model by altering the gain for the hepatic glucokinase reaction. A further attempt to improve the model was not successful (Figure 26) the conformation being similar to that of Figure 25.

It proved impossible with the present model to obtain a configuration for the intravenous glucose tolerance test curve that resembles the curve that is obtained after glucose injection in the normal human subject. In Figure S.2 can be seen superimposed upon the model produced curve (thesis Figure 25) the curve obtained when the intravenous glucose tolerance test values from 122 normal subjects are analysed and the mean plotted. Also depicted is a similarly obtained curve for the insulin response obtained during these tests (all data from Cramp, 1970). Using these curves as criteria of acceptability for the model produced curves the following observations can be made. The ten minute glucose peak is far too high as is the glucose utilisation rate (K value; see page 26). The K value for the best glucose curves produced by the model was approximately 8.5, whereas the mean K value obtained for the intravenous glucose tolerance test series mentioned above was 3.1 (Cramp, 1970). In other words, the glucose dissimilation rate appears to be two to three times faster for the model than it is in the real

physiological situation. This is to be expected with the very high initial glucose values, for the glucose utilisation rate, and hence the K value, is directly proportional to the initial elevation of the glucose concentration.

It was assumed that approximately 20 % of a glucose load would be available to the peripheral tissues while the other 80 % is disposed of by splanchnic tissues, and that the amount of glucose 'escaping' the liver determines the height of the peak of the glucose concentration curve. Indeed, that this assumption was valid has been confirmed by Wahren et al. (1974) who demonstrated that, using a combined hepatic cannulation and isotopic turnover technique, approximately 85 % of a glucose load is detained by the liver, while the remaining 15 % is available for utilisation by peripheral tissues. It is this glucose released by the liver for peripheral utilisation that determines the conformation of the glucose tolerance test curve. In the best model (Figure S.2) the ten minute glucose concentration is over twice the value found in the physiological situation, which suggests that the liver is releasing over 40 % of its load for peripheral utilisation. The insulin levels are initially high in proportion to the glucose stimulus.

The model was formulated on what was thought to be a sound enzymological basis, and it was assumed that it was inherent in the kinetics of the glucokinase/glucose-6-phosphatase reactions that glucose uptake or release would occur depending upon whether the prevailing plasma glucose concentration was high or low. It was further assumed that insulin was only involved in the hepatic control of glucose turnover by controlling the UDPG-transferase-

phosphorylase steps. It does appear from the present model that insulin could well be implicated directly in the control of hepatic glucose release, for it seems that an additional regulating step is required apart from that intrinsic to the enzyme kinetics. As was mentioned on page 9 Matsuura, Cheng and Kalant (1975) have recently demonstrated that in the rabbit glucose-6-phosphatase is controlled by insulin which acts as an inhibitor. Also, Unger (1972) and Parrilla, Goodman and Toews (1974) have suggested that the glucagon: insulin ratio may be important in controlling hepatic glucose metabolism. A two site control would not be implausible with glucagon operating at the phosphorylaæ step and insulin at the glucose-6-phosphatase step. Thus a possible reason that the present model, while incorporating what was felt to be good enzymological data, is deficient in that it does not incorporate glucose-6-phosphatase control by insulin.

Further evidence for this may this may reside in the results obtained for the simulation of the insulin injection.

Figures 32-37 represent the graphical plots of data obtained for the intravenous injection of insulin. Figure 36 shows the simulation obtained for model 12 as used for the best I.V.G.T.T. simulation. The degree of hypoglycaemia obtained was adequate when compared with that obtained in the human subject, however, the return of the plasma glucose towards the fasting level is far too rapid and unphysiological. Hepatic release of glucose may have been too rapid, but it could be possible that had a competitive inhibition of glucose-6-phosphatase by insulin been incorporated a truly physiological picture would have been obtained.

Similar conclusions could be reached over the continuous infusion of glucose.

Figures 27-31 show the graphical plots for these infusions. The initial peak is again too high (see Figure S.4) but once a steady-state is attained the hepatic autoregulatory mechanisms, probably independent of hormonal control, take over.

Some Conclusions

In the mathematical model described the importance of the fundamental structure of the biochemical system in enzymological terms has been emphasised. In this sense the model was thought to be soundly based. However, the critical weakness of the model lay in its inability to simulate absolutely the results obtained in the real physiological situation. Recent work has highlighted the possible importance of insulin as an inhibitor of glucose-6-phosphatase and it is felt that incorporation of such a mechanism would improve the model and increase its value.

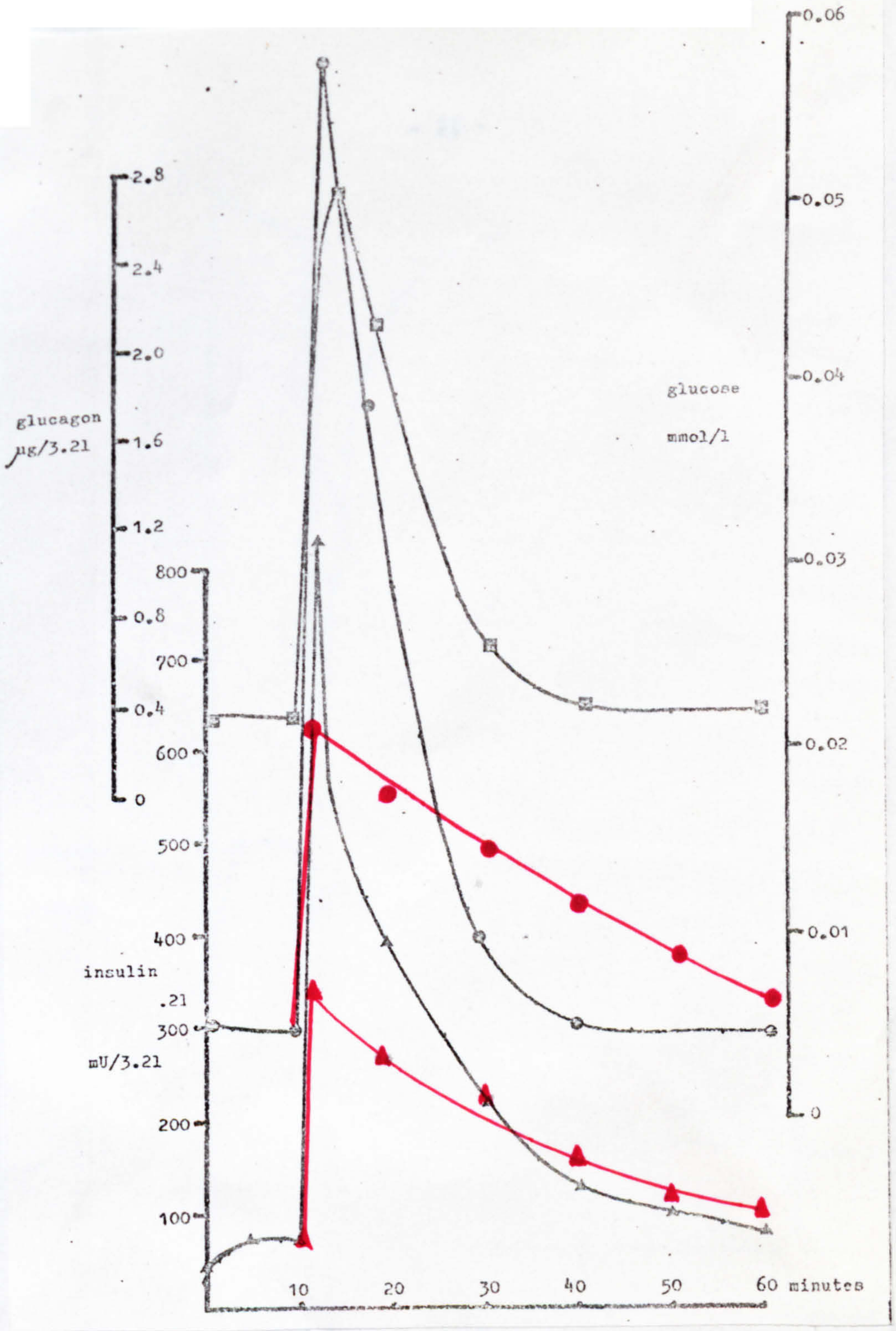

Figure S.2 Simulation of intravenous glucose tolerance test.

Superimposed (in red) upon the best obtainable model produced curves is the curve obtained from the I.V.G.T.T. glucose and insulin values from 122 normal subjects (data from Cramp, 1970). Conditions set as run 12

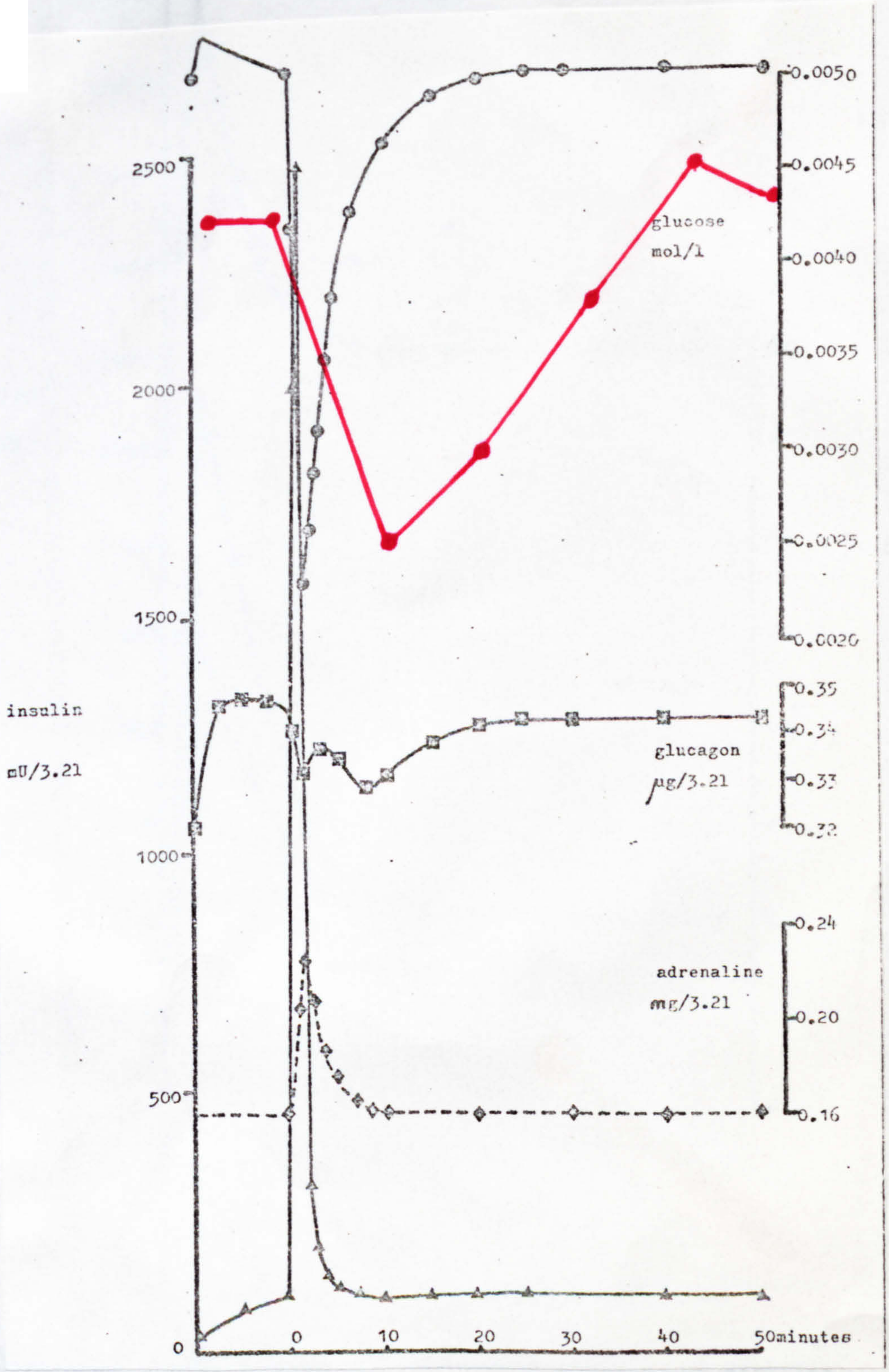

Figure S.3 Simulation of insulin injection. Superimposed (in red) is the curve obtained from an insulin sensitivity test performed upon the author

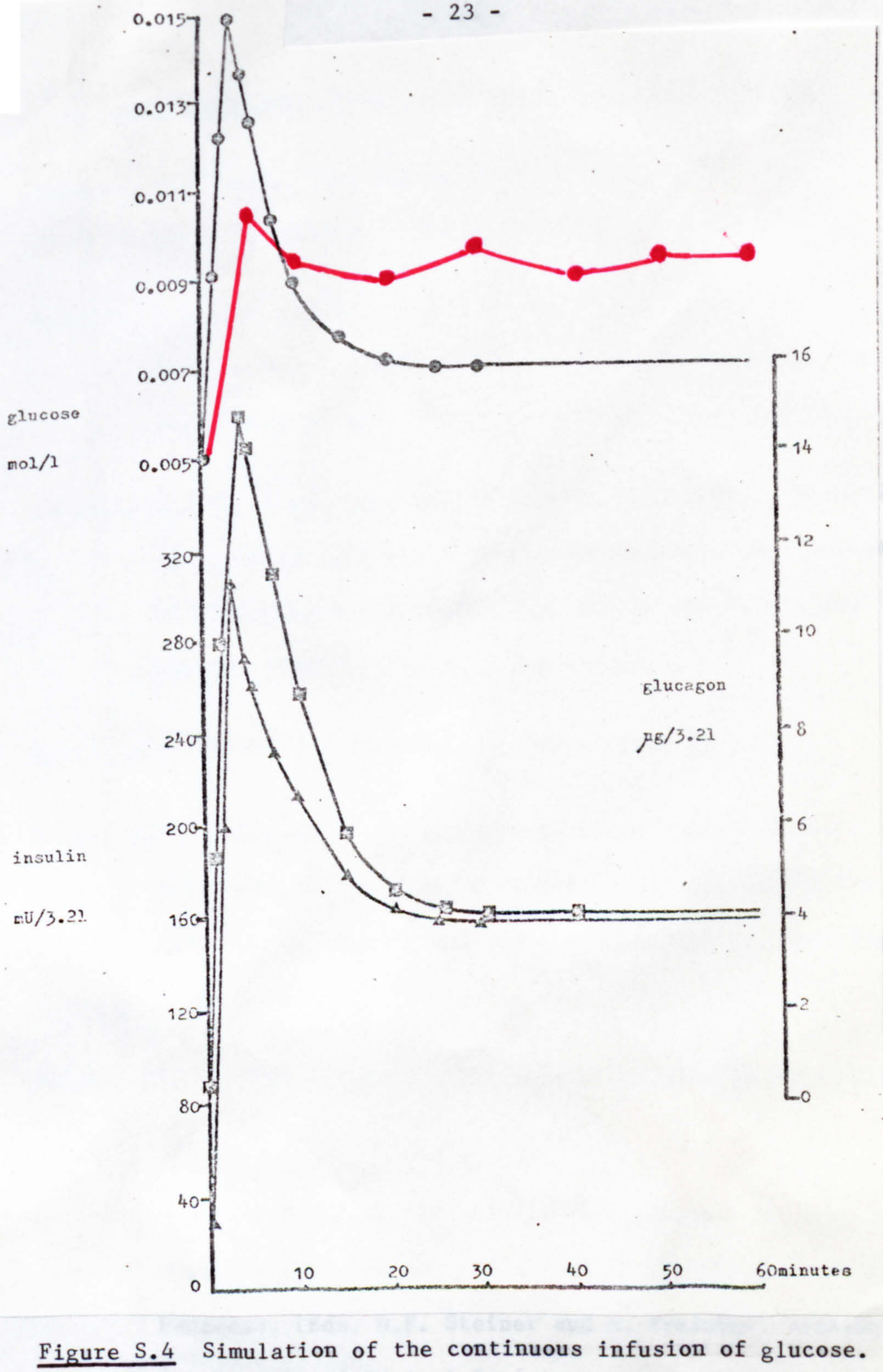

Figure S.4 Simulation of the continuous infusion of glucose. Superimposed (in red) is the curve obtained from an infusion experiment upon the author. Conditions set as run 12

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