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# Theoretical and experimental studies of factors affecting blood glucose regulation

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# THEORETICAL AND EXPERIMENTAL STUDIES OF FACTORS AFFECTING BLOOD GLUCOSE REGULATION

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# INTRODUCTION

For over one hundred years biological scientists have been investigating the metabolism, storage, and excretion of carbohydrates in the body. Exactly forty years ago the discovery of insulin served as the catalyst for the vast amount of interest that has been shown in the area of carbohydrate metabolism. On the other hand, the use of mathematical models and computers in the analysis of biological systems has only recently become feasible.

The review of literature presented in this dissertation was confined to the early work in the area of carbohydrate metabolism, the modern theories of blood glucose regulation, and the use of mathematical models in physiology. A glucose oxidase method for determining glucose concentrations in biological fluids was examined. Certain facets of glucose uptake by rat fat tissue were investigated. The coefficients of a normalized mathematical model of blood glucose regulation, using the dog as an experimental animal, were evaluated. A multi-compartment model covering some of the aspects of the complex problem of carbohydrate balance in the dog was formulated.

# REVIEW OF LITERATURE

## Early Investigations on Blood Glucose Regulation

Claude Bernard (1) in 1849 during the course of his work on glycogen discovered that damage to certain areas in the central nervous system causes an outpouring of sugar in the urine, an event which he thought occurred as a consequence of a rise in blood sugar. In 1889 von Mering and Minkowski (2) discovered that complete removal of the pancreas from dogs is followed by symptoms which closely resembled those observed in human diabetes mellitus. Early investigators established the theory that the pancreas elaborated an internal secretion which controls carbohydrate metabolism (3, 4).

By 1921 methods for measuring blood glucose and urinary glucose based on the reducing action of sugars were available. The concept of renal threshold advanced by Claude Bernard had been quantitatively measured. Moreover, the sources of blood glucose were cited as absorption from the intestine, glycogenolysis in the liver, and gluconeogenesis (from amino acids and possibly fat). In addition to this, it was stated that glucose disappears from the blood because it is oxidized in the tissues, excreted by the kidney, formed into glycogen, and converted into fat (5).

Banting and Best (6) received the Nobel prize for their work which included the preparation of an active pancreatic

extract. This contained insulin, the name for the internal secretion of the pancreas proposed by de Meyer in 1909 (7).

By 1924 conditions which produced hyper- and hypoglycemia not related to insulin levels had been noted (8). Also, differences between the action of insulin on fasted and nonfasted animals were thought to be due to the amount of glycogen stored in the liver (9). In 1926 the use of the glucose tolerance test in the diagnosis of diabetes was suggested (10). Abel, the father of pharmacology in the United States, along with his co-workers (11), isolated crystalline insulin.

By 1927 it had been noted that blood glucose disappeared faster after insulin injection in the eviscerated animal and that muscle glycogen was also increased. It had also been noted that insulin caused an increase in the rate of glucose disappearance in the isolated perfused limb (12). In the same year current theories about phlorhizin diabetes were reviewed (13). The concepts about the mechanism of phlorhizin at this time were for the most part erroneous.

In 1929 Cannon (14) discussed the homeostatic regulation of several of the body constituents, including blood sugar. His scheme for blood glucose regulation presupposed the liver to be the central storage depot. Insulin acted by increasing the storage of carbohydrates, while epinephrine caused the release of carbohydrates into the blood stream. Cannon

indicated that the release of epinephrine and insulin are, in turn, controlled by the level of the glucose in the blood.

In 1931 Cori (15) suggested that, since the blood sugar remains constant, there must exist a regulatory mechanism which is set in motion whenever the blood sugar ranges much below or much above normal. He further suggested that insulin may inhibit the enzymatic hydrolysis of glycogen in the liver. Cori, however, did admit that the fall in liver glycogen after insulin injection might be of a secondary nature. It was also established at this time that epinephrine causes a release of liver glycogen. It had also been accepted that, although muscles store glycogen, they cannot contribute to the maintenance of blood glucose in the post-absorptive state as the liver can. By this time transplantation experiments and cross perfusion experiments had shown that nervous connections with the pancreas were not necessary for regulation of blood glucose. Epinephrine release from the adrenal medulla during severe hypoglycemia had been studied. It had also been noted that there is not so much arterial-venous glucose difference in diabetics as there is in normal subjects. From this, Cori concluded that insulin has a peripheral point of attack, mainly in the muscles.

By 1934 the role of phlorhizin on renal excretion of glucose was understood with only a few investigators maintaining a mechanism of action different from our modern

concepts (16). Jensen and Evans (17) in the same year reviewed the chemistry of insulin as studies up to this time had revealed it.

By 1936 the interrelationship between galactose, fructose, glucose, and glycogen in the body had been noted. It had also been shown that the former two sugars are capable of stimulating the production of insulin but are not so potent as glucose in this respect (18).

The interrelationships between blood sugar, metabolism, and the various endocrine glands were discussed in 1936 by Houssay (19-22) in an excellent series of articles. In these articles he referred to his own work which had shown that hypophysectomized animals have an increased sensitivity to insulin.

In 1937 and 1938 Soskin and his co-workers (23, 24) performed a series of experiments which they believed established the liver as the primary organ necessary for the regulation of blood glucose.

A review by Russell (25) in 1938 indicated the effects of anterior pituitary extracts and removal of the gland were not well understood at that time. It was noted, however, that the adrenal cortex has some effect on carbohydrate metabolism (26).

By 1941 the views of Soskin (27) had received support, and he stated that the secretion of insulin was not essential

to the primary hepatic regulation of blood sugar.

In 1944 Haist (28) reviewed the factors affecting the insulin content of the pancreas. Decreases had been noted after pituitary extract administration, fasting, fat feeding, and insulin administration.

By 1945 alloxan had been used as a method for producing experimental diabetes (29, 30).

A review published in 1948 indicated that the relationship between the adrenocorticotropic hormone and the adrenal-cortical hormones was known and that some workers had questioned the validity of the concept that the liver plays an all-important role in the regulation of blood glucose (31).

Bouckaert and De Duve (32) discussed the possibility of a linear relationship between glucose utilization and blood glucose levels. They also suggested that insulin might act by stimulating a specific enzymatic system. Furthermore, they were much concerned about the hyperglycemic factor contained in insulin used for experimental purposes as well as clinical treatment (32).

Lewis (33) reviewed the attempts to prolong the action of insulin and the attempts to obtain substitutes for insulin. The latter had not been successfully achieved at that time.

At the close of the first 100 years of scientific inquiry into the physiology of carbohydrate metabolism,

Luzarow (34) reviewed the factors controlling the progression of diabetes. The presence of a hyperglycemic factor in the pancreas, now known as glucagon, was receiving considerable attention at this time.

## Modern Theories of Blood Glucose Regulation

The present degree of understanding of blood glucose regulation is well illustrated by the two passages from Turner (35). First, he says on page 180, "The blood sugar levels are held relatively constant by a regulatory system that depends largely upon the synthesis and storage of glycogen by the liver." Then, nine pages later he states, "The blood sugar level is controlled by insulin and, reciprocally, the output of insulin by the pancreas is conditioned by the blood sugar levels."

The idea that the liver is the principal organ concerned in maintaining the normal blood sugar stems from the early work of Soskin (23, 24). These studies have led to the investigation of a controversial subject, the hepatic action of insulin. There appears to be general agreement on the fact that insulin corrects disturbances in liver metabolism during states of malfunction, but it is not known with certainty whether the action is essentially a direct or an indirect one.

De Duve (36) is one of the principal advocates of significant direct action, and he reported in studies with

glucagon-free insulin that a considerable part of the disappearance of glucose affected by the hormone was attributable to an increased uptake and that conversion of glucose in the liver is comparable with that which occurs in the peripheral tissues. The work of Madison et al. (37) supported the view of De Duve.

Levine (a former co-worker of Soskin) and Fritz (38) were unable to show any action of insulin on isolated liver and, therefore, concluded that any interrelationship is either indirect or mediated through the pituitary or some hypothetical substance from a peripheral tissue. They also indicated the speed with which the body corrects for increased glucose tends to indicate that insulin secretion, and not a liver function, must be the primary regulating mechanism.

Mahler et al. (39) have presented considerable evidence which supports a concept of an indirect effect of insulin on the liver.

The data of Shoemaker et al. (40, 41) indicated that insulin failed to alter glucose output of the liver.

Van Itallie and Bergen (42) attempted to show that increased hepatic output of glucose alone is neither a practical nor a feasible way to maintain integrity of blood sugar. They also cited evidence that at blood concentrations of 150 milligrams per cent the liver continues to release glucose

into the blood.

Lang et al. (43) have reported evidence indicating the production by the liver of a humoral-factor that stimulates peripheral glucose utilization.

Another function of the liver has been suggested by Egdahl and Goldberg (44) who have provided evidence that the liver may help to regulate glucose metabolism by trapping and then releasing insulin.

Gemmil (45) in 1940 showed that insulin has a stimulatory effect on carbohydrate metabolism in the isolated rat diaphragm. In 1950 Levine et al. (46) provided evidence indicating that insulin acts by accelerating the transfer of sugar across cell membranes. Butterfield and Halling (47) observed a raised threshold for glucose in peripheral tissues of diabetics; they also found that, when the threshold was exceeded, glucose utilization proceeded normally. They stated that the threshold is reduced by the administration of insulin.

Drury and Wick (48) have suggested that any increase in oxidation of glucose is merely secondary to an increased concentration in the cell. Ball and Barrentt (49) suggested that the transport of glucose across the cell wall may be accomplished by pinocytosis. Park and Morgan (50) have shown very clearly that the transportation process involves a physico-chemical combination with some cell membrane

component and that the transportation product is not a phosphorylated compound; and they have provided evidence that insulin accelerates the transport. Gordon (51, p. 164) in 1960 stated that virtually all workers now accept the theory that insulin facilitates the transfer of hexoses across the cell membrane.

It has been assumed that glucose, once inside the cell, is phosphorylated in the diabetic as in the normal subject by hexokinase reaction; and the conversion to glycogen, pyruvate, lactate, and other 3-carbon fragments or synthesis into fatty acids can occur. However, Hall et al. (52) have indicated that an impairment in this phosphorylation may be influenced by insulin. Cori (53) postulated the function of insulin to be simply the abolishment of the inhibition of hexokinase by the pituitary and adrenal gland hormones. Zwelling (54) questioned Cori's theory on the basis of his findings which indicated hypoglycemia can be produced in the chick embryo with insulin before any of the glands are cytologically differentiated.

It has been found that an increase in the glucagon content of the pancreas follows insulin treatment in animals (55). However, as pointed out by Foa (56), this finding cannot be properly evaluated because of the lack of knowledge concerning the production and release of glucagon.

Various investigators have supplied indirect evidence

that increased insulin is liberated in the presence of hyperglycemia (57, 58). Metz (59) was able to provide direct evidence of this concept in his investigations.

Other hormones (growth hormone, adrenal steroids, glucagon, and epinephrine) have an effect on carbohydrate metabolism (60). With the exception of epinephrine, they play a permissive role in the regulation of blood glucose. That is, their rate of secretion does not alter with changes in blood sugar (61). The theory that epinephrine may be released in response to hypoglycemia stems from the early work of Cannon et al. (62). It has been questioned, however, whether epinephrine plays a role in the regulation of blood glucose levels under truly physiological conditions when drastic reductions in blood sugar do not occur (63).

Until recently diabetes and a lack of insulin were considered the same. However, there is a severe histological decrease in the number of beta cells in only a minority of diabetic patients, mainly the younger ones. In fact, with the most sensitive of present techniques the pancreas of 25 per cent of these patients show no significant pathological findings (64, 65). Mirsky and Brohkahn (66) demonstrated that rat liver extracts and rat liver slices both contain an insulin inactivating system (insulinase); in the light of the histological studies, the interesting possibility is raised that certain abnormalities might be caused by changes in

insulinase activity. Mirsky and Perisutti (67) have shown that the liver also contains an insulinase-inhibitor preparation that effectively inhibits the destruction of insulin. In addition to this insulin antagonist, Field (68) has detected a circulating insulin antagonist that does not seem to be a known hormone or insulinase but is a protein. Haist (69) stated that he feels that it is unlikely that diabetes is due basically to a primary reduction in insulin synthesis.

As far as the action of insulin on peripheral tissues goes, one important feature seems to be the ability of the hormone to bind itself to these tissues. Stadie et al. (70) reported that this bound insulin is the active form of insulin. Haugaard and Marsh (71) have confirmed that this binding occurs in adipose tissue as well as in muscle. This opens up the possibility of still another type of insulin antagonism which is neither insulinase nor that reported by Field.

In 1960 Stetten and Stetten (72) supplied evidence that the breakdown of glycogen and the route of synthesis are not the same. They stated that the breakdown is predominantly by the phosphorylase pathway, while the uridinediphosphoglucose route is the major pathway of synthesis.

From the clinical point of view, perhaps the significant achievement in the period from 1949 to 1961 is the development of oral hypoglycemic agents. These agents have led to

attempts to develop methods for measuring insulin in body fluids (51, p. 164, 73).

The work of Sanger and Tuppy (74), who determined the structure of insulin, was a significant chemical achievement and may, in the near future, lead to a better understanding of the physiological mechanism involved in the regulation of blood glucose.

## Mathematical Models of Biological Systems

For some time mathematics and physics have been interrelated disciplines. However, only within the last three decades has there been any attempt to couple mathematics and the biological sciences. The idea of building mathematical models seems to have originated with Lotka (75). Rashevsky (76), founder of the Bulletin of Mathematical Biophysics, in his book Mathematical Biophysics: Physico-mathematical Foundations of Biology treated such topics as mathematical theories of cell respiration, cell division, cell polarity, various aspects of neurophysiology such as reaction times, discrimination, thinking, aesthetic perception, abstraction, and learning.

In 1948 Wiener (77) postulated that many of the physiological processes of the body were controlled by mechanisms analogous to the electronic concept of feedback. Among the possible factors controlled by this type of mechanism, Wiener listed: nervous regulation of posture, body temperature,

leucocyte production, heart rate, blood pressure, sexual cycle, calcium metabolism, and, in general, all of the mechanisms of homeostasis.

The use of mathematical models of biological systems involving differential equations, with or without the aid of analog computers, was given encouragement by Chance et al. (78, 79) who in 1952 developed a set of differential equations dealing with the kinetics of catalase action. Their work showed that the results of programming these equations on an analog computer and the experimental results concerning the disappearance of the catalase-hydrogen peroxide complex were in close agreement. Yang (80) in 1955 and Chance et al. (81) in 1962 have suggested further ways in which analog and digital computer representation of biochemical processes can be used to advantage.

In 1954 Grodins et al. (82) developed a set of differential equations dealing with the dynamics of homeostatic respiratory regulation. Since then, several other reports offering different mathematical models of respiratory regulation have been presented (83-85).

Various phases of the complex cardiovascular system have been subjected to mathematical analysis with and without the aid of computers (86-88).

Hardy (89) used differential equations in describing the regulation of the body temperature by sweating,

vasomotor action, and metabolic rate.

Several mathematical models have been offered to simulate the changes in the various compartments of the body after administration of tracers (90-95). The work done at the research laboratories of the Upjohn Company (96, 97) is different from other reports in that the experiments were designed for computer simulation of the changes in various compartments of the body. The rate of absorption, the rate of renal excretion, the blood levels, and the rate of binding or storage in the kidney were investigated. Although the drug used was not metabolized, the authors concluded that a simple physiological model could be formulated and investigated to advantage by use of differential equations and analog techniques.

Another mathematical approach to physiological regulation was reported by Stark and Cornsweet (98) who applied servomechanism techniques to studies of the pupillary reflex.

Several of the endocrine systems have also been subjected to mathematical investigation. Brownell et al. (91) offered a mathematical model of the interrelationship of the thyroid and iodine. Danzinger and Elmergreen (99) reported a mathematical investigation of the thyroid pituitary homeostatic mechanism. Roston (100) has presented mathematical models of the calcium, inorganic phosphate, and parathyroid

system; and of the sodium, water, aldosterone, and anti-diuretic hormone complex; as well as having considered the thyroid-pituitary axis.

The regulation of blood glucose has also been subjected to mathematical investigation. A mathematical treatment of the interrelationship of glucose and insulin has been presented by Bolie (101, 102). The effect of an intravenous injection of glucose received mathematical consideration by Ikkos and Luft (103, 104), while Wajchenberg et al. (105) presented a mathematical model for glucagon-induced hepatic glycogenolysis.

By 1961 the field of mathematical models, accompanied by the use of computers in physiology, had generated sufficient interest that the Federation of American Societies for Experimental Biology presented a symposium dealing with this subject. The papers presented there ranged from an introduction to the principles of digital and analog computers to the specialized topic of computer simulation of neuron-like nets (106-110).

## DETERMINATION OF GLUCOSE IN BIOLOGICAL FLUIDS

In this investigation glucose levels in blood and other fluids were determined by a modification of the method developed by Keilin and Hartree (111). This method utilizes an enzyme, glucose oxidase, and is reported to be more specific for glucose than the reduction methods that have been used for many years. Several variations in this method and different applications of it have been reported (112-120).

This method is based on the following simplified scheme of reactions:

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{oxidase}]{\text{Glucose}} H_2O_2 + \text{gluconic acid}$$

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{Peroxidase}} H_2O + \text{oxidized chromogen}$$

A mixture of the two enzymes and oxygen acceptor is marketed in the U. S. A. under the proprietary name of "Glucostat" by the Worthington Biochemical Corporation, Freehold, New Jersey.

### Materials and Methods

In accord with the work of Huggett and Nixon (114), anticoagulation and inhibition of glycolysis were achieved with a mixture of sodium fluoride and potassium oxalate, 10 mg. and 30 mg., respectively, per 1.0 ml. of blood.

Huggett and Nixon report a tenfold increase in the concentration of this mixture to be without effect on the reagents used. In addition, samples of blood or other fluids to be kept overnight were stored at $0^{\circ}$ C.

"Glucostat" was prepared for the micromethod as described by others (119), except that a pH 7 phosphate buffer was used instead of distilled water. To 2.0 ml. of $H_2O$, 0.1 ml. of blood or artificial serum (Krebs bicarbonate solution with glucose and gelatin) was added. To this, 1.0 ml. of 2.0 per cent $ZnSO_4 \cdot 7H_2O$ and 1.0 ml. of 1.8 per cent $Ba(OH)_2 \cdot 8H_2O$ were added. This was mixed and centrifuged. Glucose standards and blanks were prepared in a similar manner. To 2.0 ml. of the filtrate, 2.0 ml. of the "Glucostat" reagent were added. This was allowed to stand at room temperature for 60 minutes. Then one drop of 6N HCl was added to stop the reaction and stabilize the color. The tubes were allowed to stand for 5 minutes, and then the solutions were read at 400 mu on a spectrophotometer. The blank was used to adjust the optical density reading to zero.

Duplicate determinations were done on all samples and averaged. A maximum variation of .02 (optical density) for blood samples and .01 (optical density) for artificial serum samples was allowed. Pairs of readings with greater variation were discarded and a new pair of determinations repeated.

The concentration of glucose was calculated as follows:

$$\frac{\text{O.D.(unknown)}}{\text{O.D.(standard)}} \times \text{Concentration(standard)} = \text{Concentration (unknown)}$$

## Results and Discussion

### Influence of buffer on optical density

The optical density on a set of glucose standards diluted to contain 25, 50, 75, 100, 125, and 150 milligrams per cent of glucose was determined using the above method. Similar determinations were made using the same method except that distilled water as indicated by Worthington Biochemical Corporation (119) was used instead of pH 7 buffer.

The results of these procedures are shown in figure 1. The buffered reagent gave linear results over the range 25 to 150 milligrams per cent of glucose, while the optical density of the non-buffered reagent was nonlinear in samples containing more than 75 milligrams per cent glucose.

### Reproducibility

Ten samples each of a 100 milligrams per cent glucose standard, dog blood, and a Krebs-bicarbonate solution with glucose and gelatin were used; the optical density of the first and the concentrations of glucose in the other two were determined. The results of this are shown in table 1.

The smallness of the variation shown in table 1 confirms

the conclusions of other investigators that this can be used to determine the blood glucose level. Munck (120) has used this method for determination of glucose levels in Krebs-bicarbonate with glucose and gelatin added. The results shown in table 1 confirm the assumption of Munck that the contents of this solution do not interfere with the reproducibility of this method for determining glucose concentration.

Effect of temperature

With some preparations of this enzymatic reagent inhibition of color due to trace enzymes in samples that are incubated at higher temperatures over long periods of time has been reported (119). The results of optical density determinations on 100 milligrams per cent glucose samples incubated at room temperature (22° C.) and at 37° C. are shown in figure 2 and support these findings.

## ADIPOSE TISSUE UPTAKE OF GLUCOSE

In 1960 at the beginning of these studies it was hoped that blood insulin concentration could be measured with sufficient accuracy so that the change in blood levels of insulin could be determined repeatedly during an infusion of glucose or insulin. Preliminary investigation of various techniques indicated that none of the methods available were suitable for this purpose because of a lack of precision. A reliable and accurate method for the estimation of the insulin concentration in the blood would certainly prove useful for the investigation of many problems involving carbohydrate metabolism. Three recent reviews of this problem provide excellent discussion of the various methods that have been used for estimating the insulin concentration in blood. All these authors state that the methods presently available lack precision (73, 121, pp. 1-137, 122).

One of the methods devised for measuring insulin plasma levels depends on the hypoglycemic effect of insulin in rats. The test animals used had been prepared in different ways to increase their sensitivity. Gellhorn et al. (123) used hypophysectomized-adrenalectomized rats and found that 200 microunits of insulin produced a small but significant lowering of blood sugar in these animals. This method was improved by giving the rats alloxan (124). The technical

difficulties involved in the preparation and maintenance of these animals were considerable.

Another approach using isolated tissues has been tried. One of these methods depends on the fact that small amounts of insulin increase the conversion of glucose to glycogen in the isolated rat diaphragm. By controlling experimental conditions this method can be used for estimation of plasma insulin-like activity (the sum of insulin and its synergists and antagonists) (125-127).

Another *in vitro* method having much greater sensitivity is based on increase utilization of glucose by rat epididymal fat pads. The uptake of glucose (128), the production of carbon dioxide (129), and the production of isotopic labeled carbon dioxide from labeled glucose (130) have been used as measures of this increased utilization. Renold *et al*. (130, 131) reported that adipose tissue is responsive to many substances at high concentrations, including some of the hormones. However, they note that this preparation is sensitive to insulin at levels equal to, or less than, physiological concentrations.

Still another assay is the immunological method developed by Yalow and Berson (132). This is based on the production of anti-serum to human insulin by the guinea pig and an electrophoretic separation of radioactive insulin to which the antibodies are bound. While the immunoassay is somewhat

more precise in experiments measuring human plasma insulin it necessitates some rather costly equipment not readily available. Furthermore, since the feasibility of measuring insulin concentration in dog serum was one of the preliminary considerations of this investigation, the adaptation of the immunoassay method to the measurement of insulin concentration in dog plasma would require additional proof that dog insulin does closely follow electrophoretically the behavior of human insulin. Moreover, the antibodies produced in the guinea pig by dog insulin, as compared to the antibodies produced by human insulin, must have a similar binding capacity.

All of these methods are very sensitive, especially when it is considered that crystalline insulin contains 24 units per milligram. Furthermore, the best estimates for the physiological range of insulin concentrations in the blood are from about 0.05 to 1.0 milliunit per milliliter. With this small amount of insulin present in the blood, existing assays, all of which lack precision, are unsuitable for experimental use but have been applied to clinical situations (73, 121, pp. 1-137, 133).

It was felt that the use of adipose tissue as an in vitro preparation for studying factors affecting insulin antagonism and synergism might prove an interesting area of investigation. Many aspects of carbohydrate metabolism in adipose tissue have not yet been investigated. Thus, the

following studies were undertaken with the idea that results obtained might contribute to the eventual development of a satisfactory insulin assay technique utilizing the rat adipose tissue.

## Materials and Methods

The glucose uptake of rat epididymal fat pad tissue under various conditions was studied by using a slight modification of the technique described by Beigelman and Onoprienko (134).

A total of 124 male Sprague Dawley rats weighing from 125 to 195 grams was used. Before experimental use the rats were housed 4 to a cage and given Purina laboratory chow and water ad libitum. They were sacrificed by a blow on the head. Laparotomy was performed and the testicular epididymal fat swiftly excised. Segments of this tissue, weighing generally from 40 to 70 milligrams, were quickly cut, weighed, and incubated in Krebs-bicarbonate buffer solutions containing glucose (1.00 to 1.30 milligram/milliliter), gelatin (1 milligram/milliliter), and various concentrations of insulin. The incubation proceeded for 6 hours at 37°c. under an atmosphere of oxygen (95%) and carbon dioxide (5%) in a Dubnoff Metabolic shaker. The gas was introduced on commencing the run, and again midway through the run, into the chamber at a rate of about 2 liters/minute for 10 to 15

minutes. Throughout the incubation period the gas mixture was allowed to leak slowly into the chamber at a minimal flow rate. The shaker was run at 75 revolutions/minute.

The stock insulin solution was made by dissolving recrystallized glucagon-free insulin (Eli Lilly) in a concentration of 1 milligram/milliliter of distilled water; 1 N HCl was added slowly, dropwise with mixing, until the insulin was completely dissolved. The final pH was about 3 to 4. The stock solution was kept either in the refrigerator or in the frozen state for a period which did not exceed 3 months. Appropriate dilutions with Krebs-bicarbonate solution were made each day (134-136).

In any series of determinations several blanks were incubated. These contained the solution but no adipose tissue. Glucose determinations were made in the manner previously described. The difference between the glucose in the blanks and in the solution containing the adipose tissue constituted the glucose uptake. This was expressed in milligrams of glucose per gram of tissue per hour.

The rats were divided into groups. Tissues from 5 rats were incubated in the following insulin concentrations: 0.1, 0.25, 0.5, 0.75, and 1.0 unit/milliliter. The glucose uptake was determined in the manner described above for 2 pieces of tissue from each rat, at each of the 5 insulin levels.

Fat tissues from another group of rats were cut into

various sizes with a weight range from less than 10 to 100 milligrams. These tissues were incubated in Krebs-bicarbonate medium containing an insulin level of 0.5 milliunit/milliliter. The glucose uptake of every tissue segment from each rat was determined after incubation as previously described.

Tissues from a third group of 7 rats were incubated in Krebs-bicarbonate medium containing 0.25 milliunit/milliliter of insulin. Ten pieces of tissue were taken from each rat; and 5 were incubated in the above solution, while the other 5 were incubated in the same medium except that 0.03125 milligram/milliliter of hexamethonium, present as the bromide, had been added. Glucose uptake after incubation was determined as previously described.

The remaining rats were paired on the basis of weight prior to treatment. An attempt was made to keep the tissue size within a 10 milligram range in each pair of rats, while minimizing extra handling of the tissue. Five to 7 pieces of tissue were taken from each rat and incubated in Krebs-bicarbonate solution with an insulin concentration of 0.25 milliunit/milliliter. Tissues from pairs of rats were incubated at the same time, and the solution used for incubation of tissues from each pair of rats was exactly the same. Glucose uptake on each piece of tissue was determined as before.

Eight hours before sacrifice food was removed from 1 member of each pair of rats. The fasted rats were allowed free access to water. The non-fasted animals were allowed free access to both food and water. Twelve rats were in this group.

In another group of 12 paired rats half were injected intraperitoneally 4 hours prior to sacrifice with 10 milligrams/kilogram of choline chloride in a saline solution. The volume of injection was less than 2.0 milliliters of solution. The control animal of each pair of rats was injected in a similar manner with a like volume of saline.

In the next group of 18 paired animals tetraethyl ammonium, present as the chloride, at a dosage of 5 milligrams/kilogram was injected intraperitoneally 30 minutes before sacrifice into half of the rats. The other member of each pair received an appropriate volume of saline.

In another group of 18 paired rats half received 20 milligrams/kilogram of hexamethonium, present as the bromide, by the intraperitoneal route 1 hour prior to sacrifice. The other half of each pair received an injection of saline of the appropriate volume.

In another group of 18 paired animals similar treatment was given. Tissues from this group were incubated in Krebs-bicarbonate medium containing no insulin.

## Results and Discussion

### Variation of individual rats

The data for the analysis of variance shown in table 2 were obtained by measuring the glucose uptake (milligram of glucose per gram of tissue) from 24 rats injected with saline 1 hour prior to sacrifice. Six pieces of epididymal fat tissue from each of these rats were incubated in Krebs-bicarbonate medium containing 0.2 milliunit/milliliter of insulin. The variation from rat to rat was significantly greater than the variation from tissue to tissue within each rat. Beigelman (128, 133) stated that individual rats appear to present inherent variation in fat pad glucose uptake in response to insulin. The above findings provide statistical confirmation for his generalization.

In figure 3 is shown the relation between the body weight of rats and glucose uptake by their adipose tissue in response to an insulin level of 0.2 milliunit/milligram. These results from 46 rats weighing from 149 to 195 grams indicate that there is a linear decrease in the glucose uptake of epididymal fat as the body weight increases ($Y = 6.35 (-0.0295) X$; $r = 0.825$). Extension of the regression line indicates that at a body weight of about 215 grams no sensitivity to insulin should be observed. These findings are in agreement with reports which indicate that tissue from

animals in the weight range 100 to 200 grams had considerably higher glucose uptake than tissue from heavier animals and that adipose tissue sensitivity to the lower levels of insulin disappears in some rats weighing more than 220 grams (128).

The results of wide variation of tissue size from individual rats on glucose uptake response at an insulin level of 0.5 milliunit of insulin are shown in figures 4 through 7. These findings support the previous observation on groups of rats that there is no significant difference in glucose uptake of tissue weighing less than 100 milligrams (128).

Figure 8 and 9 show the variation in glucose uptake of tissue with a smaller variation in weight. Some of these tissues were cut into at least 7 smaller pieces, and even this heroic treatment did not seem to alter the total glucose uptake of adipose tissue.

Figures 10 through 15 show the effect of variations in concentration of insulin within the physiological ranges on rat adipose tissue glucose uptake. Within each rat a trend to increased uptake with increasing insulin levels was observed. These findings are consistent with the findings of others who, using labeled carbon dioxide, reported linearity at insulin concentrations in the physiological range (131). However, other authors using a wider range of insulin concentration have noted a relative decrease in insulin uptake

with higher levels of insulin concentration in the Krebs-bicarbonate medium (128, 136, 137).

The above findings serve as a basis for pairing rats by weight in subsequent investigations in which other factors affecting glucose uptake of rat adipose tissue were studied. Differences in weight seem to be a main factor to which variation between rats may be related. However, pairing will also eliminate variations in the glucose uptake due to environmental differences and slight variations in the composition of Krebs-bicarbonate medium. For example, it has been shown that there is no statistical difference between incubation of tissues from different groups of rats in a solution containing less than 80, 80 to 104, and 104 to 140 milligrams per cent glucose and 0.1 milliunit/milliliter of insulin. However, such variation in glucose concentration might contribute to the variation between rats. Likewise, slight differences in ionic composition, while not significantly affecting glucose uptake between groups of rats, might contribute to variation between individual rats (138, 139). These differences would be eliminated by pairing.

Nutritional effects

Lipogenesis is influenced by the diet. It is diminished during fasting and becomes very active when starved animals are refed with a high carbohydrate diet. This increased

lipogenesis upon refeeding has been associated with increased activity in the liver of the intact animal (140, 141).

A similar relationship between the nutritional state and lipogenesis has been found by using isolated fat tissue (137, 142). The amount of decrease in fasting rats 24 hours prior to measuring the fat tissue uptake in the presence of insulin is small and in some cases, not statistically significant.

The effect of an 8 hour fast on tissue glucose uptake at an insulin level of 0.25 milliunit/milliliter is shown in table 3. Only a small, insignificant decrease was noted.

Choline is a growth factor concerned especially with the transport and deposition of fat. Deficiency produces marked accumulation of fat in the liver. Administration of choline reduces the accumulation of fat in the liver while increasing the storage of fat in the peripheral tissue. Its site of action is presumed to be in the liver and its mechanism of action related to the conversion of fat in the liver to phospholipids. The formation of phospholipids after injection of radioactive phosphate is increased in the livers of rats by a single dose of choline (143, 144).

In the present studies no statistically significant change in the glucose uptake of rat adipose tissue at an insulin concentration of 0.25 milliunit/milliliter was observed 4 hours after the rats were treated with 10 milligrams/

kilogram of choline chloride. See table 2.

Since the rate of metabolism in rats is high, an 8 hour fast in these animals may be equal in many respects to a much longer fasting period in other species, for example, man or dog. Therefore, it is felt that the above data indicate the effect on the maintenance of the blood sugar level of both fasting and choline has as its site of action some organ other than the peripheral adipose tissue.

Effect of ganglionic blocking agents

The previous work dealing with the in vitro effects of epinephrine is controversial. The addition of epinephrine to the incubation medium has been reported by several authors to increase the activity of adipose tissue (131, 145). Epinephrine produces this effect both alone and in the presence of insulin. The effect of epinephrine and insulin together was, however, less than the total increase when used separately. On the other hand, Hagen and Ball (146) have shown that epinephrine (10 micrograms/milliliter) reduces the net carbon dioxide and lactic acid production. When epinephrine was injected into the rat prior to use, no effect was noted. This was presumably because of the rapid rate of destruction of epinephrine (145).

If epinephrine does enhance the uptake of glucose by adipose tissue, it would certainly be unexpected in view of

the general inhibitory effect of epinephrine upon peripheral glucose utilization in the intact animal (147). Furthermore, it has been well established that epinephrine has an inhibitory effect on the glucose uptake of the isolated rat diaphragm (148).

In the present studies ganglionic blocking agents were used to investigate the effects of epinephrine on adipose tissue glucose utilization. Hexamethonium and tetraethyl ammonium block the transmission of impulses across the autonomic ganglion without depolarizing the cell bodies. They do not prevent the release of acetylcholine from the preganglionic nerves, but they prevent the stimulating effects of acetylcholine or other ganglionic stimulating compounds (149, 150). The adrenal medulla is innervated by preganglionic sympathetic fibers, and its secretory cells are in many ways analogous to postganglionic neurons (151). Ganglionic blocking drugs inhibit the release of epinephrine and norepinephrine from both the adrenal medulla and the adrenergic sympathetic postganglionic neurons (152). Both of the ganglionic agents, hexamethonium and tetraethyl ammonium, have a rapid action. Because of the rapid destruction of epinephrine its blood level is soon greatly lowered after hexamethonium and tetraethyl ammonium are administered (153).

The results obtained in the present study are shown in table 2. Administration of hexamethonium and tetraethyl

ammonium prior to sacrifice increased significantly the uptake of the glucose by the rat adipose tissue in the presence of insulin (0.25 milliunit/milliliter). The uptake of glucose by this tissue was not significantly increased when hexamethonium was administered in the same manner, but no insulin was in the incubating medium. Furthermore, addition of hexamethonium to the medium did not significantly increase the uptake of glucose in the presence of insulin.

This data indicates that hexamethonium, presumably by reducing the epinephrine levels, increases the glucose uptake by adipose tissue in the presence of insulin. This finding supports the theory that epinephrine has an antagonistic action on the peripheral effect of insulin (63, 147).

## EVALUATION OF A SIMPLE MODEL

A simple model of blood glucose homeostasis involving a pair of differential equations was presented by Bolie (102). In their normalized form these equations are as follows:

$$\frac{dx}{dt} = p - \alpha x + \beta y \qquad (1)$$

$$\frac{dy}{dt} = q - \gamma x - \delta y \qquad (2)$$

The deviations in insulin and glucose concentrations from their normal values are represented respectively by x (unit/liter) and y (gram/liter). The normalized rate of insulin and rate of glucose infusion are denoted by p (unit/hour/liter) and by q (gram/hour/liter). The regulatory coefficients represented by alpha, beta, gamma, and delta are respectively the normalized decay constant for insulin ($hour^{-1}$), the normalized sensitivity of pancreatic insulin output rate to the blood level of glucose (unit/hour/gram), the combined and normalized sensitivity of liver glycogen storage rate and tissue glucose utilization rate to elevated insulin concentration (gram/hour/unit), and the combined and normalized sensitivity of liver glycogen storate rate and tissue glucose utilization rate to elevated glucose concentration ($hour^{-1}$). In the paper cited, these coefficients ($\alpha$ , $\beta$ , $\gamma$ , $\delta$ ) were estimated from the works of other

authors, by several methods. Using the steady state conditions found in normal animals, the following estimates were made: $\alpha$ = 0.916, $\beta$ = 0.198, $\gamma$ = 3.23, and $\delta$ = 3.04. By considering experiments dealing with specific organ responses, the following estimates of the coefficients were obtained: $\alpha$ = 0.625, $\beta$ = 0.125, $\gamma$ = 5.00, and $\delta$ = 2.52. By use of transient characteristics of the intact animal the following estimates were reported: $\alpha$ = 0.800, $\beta$ = 0.300, $\gamma$ = 4.80, and $\delta$ = 3.20.

It was felt that an estimate of alpha obtained under the experimental conditions of this investigation would be more applicable than estimates for the destruction rate of insulin in other preparations. Haugaard et al. (154), in dogs anesthetized with pentobarbital (which is the same preparation used in the present investigation), estimated the biological half-life of insulin as 50 to 55 minutes. By assuming a first-order chemical reaction for the disappearance of hormones from the blood and by using the data from Haugaard et al., alpha was calculated to be in the range of 0.75 to 0.83 $hour^{-1}$. These values were close to the estimates of 0.8 obtained by Bolie.

By using this estimate of alpha, subsequent investigations were undertaken to evaluate the other coefficients presented by Bolie.

## Materials and Methods

A total of 56 mongrel dogs of either sex, weighing from 5 to 24 kilograms was used. Before experimental use the dogs were housed 1 or 2 to a cage and given General Mills SureChamp Meal ticket dog food and water ad libitum. Food was removed 24 hours before the animals were to be used. All insulin used in these experiments was glucagon-free insulin supplied by the Eli Lilly Company. Sterile glucose solutions supplied by the Fort Dodge Laboratories were used. All the animals were anesthetized with sodium pentobarbital, and the femoral vein and artery were cannulated.

In 3 animals a constant infusion of saline at the rate of 22 milliliters/minute was begun after they had been anesthetized for 35 to 50 minutes. Blood samples of 1 milliliter were obtained from the femoral artery before the start of infusion and every 15 minutes thereafter for 2 hours. The glucose concentration in these samples was determined by the method previously described.

Four other groups of animals, containing 10, 11, 10, and 9 dogs, were treated in a similar manner except that they were infused with a saline solution containing glucose or insulin in such concentration that they received 0.25 gram/hour/kilogram or 0.5 gram/hour/kilogram of glucose or 0.025 unit/hour/kilogram or 0.05 unit/hour/kilogram of insulin respectively. In some instances the infusion and sampling

continued for periods in excess of 2 hours.

A group of 3 dogs was injected with 0.1 gram/kilogram of glucose over a 10 minute period, and blood samples taken before the start of the injection and at 10, 30, and every 10 minutes thereafter. The blood glucose levels were determined as previously described. Another group of 3 animals was treated in a similar manner except the dosage of glucose was 0.25 gram/kilogram.

Three dogs were prepared as described previously; then 1 milligram/kilogram of atropine was administered intravenously. Carotid blood pressure and a continuous electrocardiogram were recorded by use of a Physiograph (E & M Instrument Company). Thirty minutes after the injection of atropine 0.1 unit/kilogram of insulin was injected intravenously. Blood samples were taken periodically and glucose levels determined as before. Another group of 3 animals was treated as above, except at 45 minutes after the injection of insulin an infusion of epinephrine was started. The infusion rate was controlled so that a 10 millimeters of mercury increase in blood pressure was maintained. The rate of epinephrine infusion varied from $10^{-8}$ to $10^{-7}$ gram/kilogram/minute within each dog.

Four dogs were infused with a combination of both glucose and insulin. Two received glucose at a rate of 0.25 gram/hour/kilogram and insulin at a rate of 0.11 unit/hour/

kilogram. The other 2 dogs were infused with a combination of glucose and insulin at rates of 0.25 gram and 0.04 unit per hour per kilogram. Blood samples were collected, and glucose determinations were performed in the manner previously described.

## Results and Discussion

### Estimates of coefficients

Table 5 and figures 15 through 18 show the changes in blood glucose observed during the infusion of insulin or glucose. The mean maximum increase in the animals infused with 0.25 gram/hour/kilogram was 17 milligrams per cent glucose, which occurred at 30 minutes after the start of infusion. A steady state 2 milligrams per cent higher than the initial blood sugar level was established after 60 minutes. At a higher dose level of 0.5 gram/hour/kilogram a maximum rise in blood glucose of 30 milligrams per cent was observed at 45 minutes after the start of the infusion. A steady state blood sugar level was established at 105 minutes following the start of the infusion. This level was 12 milligrams per cent higher than the initial level. During the infusion of insulin at rates of 0.025 and 0.05 unit/hour/kilogram, a steady state was obtained at about 135 minutes after the start of the infusion. These levels were about 10 milligrams per cent and 30 milligrams per cent of glucose

below the initial levels.

When the glucose levels have reached a steady state, equations 1 and 2 may be represented by the following equations,

$$p = \alpha x - \beta y \tag{3}$$

$$q = \gamma x \quad \delta y, \tag{4}$$

since the rate of change of glucose is zero and the rate of change in insulin may be assumed to be zero (155).

If only glucose is infused, and equation (3) is multiplied by $\gamma$ and equation (4) by $\alpha$,

$$0 = \gamma\alpha x - \gamma\beta y \tag{5}$$

$$\alpha q = \alpha\gamma x + \alpha\delta y \tag{6}$$

which solved simultaneously for y give

$$\alpha q = y\,(\alpha\delta + \beta\gamma\,). \tag{7}$$

In a similar manner, during an infusion of insulin equations (3) and (4) may be represented by

$$\gamma p = -y\,(\,\alpha\delta + \beta\gamma\,). \tag{8}$$

Since y in equation (7) represents the change from normal during glucose infusion and the y in equation (8) represents a similar change during insulin infusion, they will be denoted by $y_g$ and $y_i$. By combining equations (7) and (8) it is found that

$$\frac{y_g}{y_i} = \frac{q\alpha}{p\gamma} \tag{9}$$

or

$$\gamma = \frac{\alpha q y_i}{p y_g} . \tag{10}$$

By substituting the following values into equation (10):

$\alpha$ = 0.80 hour$^{-1}$

q = 1.00 gram/hour/liter $\quad$ $y_i$ = 0.12 gram/liter

p = 0.10 unit/hour/liter $\quad$ $y_g$ = 0.02 gram/liter

it is found that $\gamma$ = 4.8 grams/hour/unit. The values shown above were obtained by conversion of infusion rates and corresponding results into the units listed. Additional estimates of gamma were obtained in a similar manner using the other infusion rates; and results were 5.16, 2.12, and 1.75 grams/hour/unit. The values of 5.16 and 4.8 for gamma are closest to the estimate of 4.8 given by Bolie. The lower estimates involve results obtained by infusing 0.5 gram/hour/kilogram of insulin.

By balancing the effects of an infusion of insulin and glucose on glucose deviation, equation (10), when given proper values for gamma and alpha, predicts that a certain rate of glucose infusion requires a certain rate of insulin infusion to maintain minimal deviation in blood glucose levels:

$$\gamma p = \alpha q. \tag{11}$$

By using $\alpha = 0.8$ hour$^{-1}$ and $\gamma = 5.0$ grams/hour/unit, it was determined that a combination of 0.25 gram/hour/kilogram of glucose and 0.04 unit/hour/kilogram should maintain the blood sugar at a relatively constant level. The results of infusion of insulin and glucose at these rates are shown in figure 19. The blood sugar of these animals remained relatively constant, although in one dog an initial drop of 10 milligrams per cent glucose was noted.

By using $\alpha = 0.8$ hour$^{-1}$ and $\gamma = 2.0$ grams/hour/unit, it was determined that a combination of 0.25 gram/hour/kilogram and 0.11 unit/hour/kilogram should maintain the blood sugar at a relatively constant level (equation 11). The results of infusing a combination of glucose and insulin at these rates is shown in figure 20. The blood sugar level of these animals did not remain constant and showed a lowering trend.

Best (156) reported that blood sugar has been kept at a normal level in dogs by infusing both insulin and glucose. When the insulin infusion rate was between 0.06 and 0.4 unit/hour/kilogram, the corresponding glucose requirement was between 0.2 and 6.0 grams/hour/kilogram. The lower values were obtained in anesthetized dogs. Glucagon-free insulin was not used in the experiments reported by Best.

It is felt that the results of the present study, along with those reported by Best, indicate that 5.0 grams/hour/

unit is a reasonable estimate for the value of gamma.

Metz (59), using fasted dogs anesthetized with pentobarbital, has shown that the pancreatic output of insulin approaches zero as the blood sugar is lowered. Metz used the rat diaphragm method to estimate insulin concentration of the blood in the pancreatic venous blood. This method is reported to give somewhat higher results than newer techniques (121). Furthermore, the liver reduces the effective insulin concentration in the blood, and the pancreatic venous blood passes through the liver (157).

On the basis of the above it is reasonable to assume that at lowered blood glucose levels the insulin output of the pancreas is insignificant in comparison with a reasonably high insulin infusion rate. Thus, equations (3) and (4) may be modified as follows:

$$p = \alpha x - 0 \tag{12}$$

$$0 = \gamma x + \delta y. \tag{13}$$

This pair of equations then give the solution,

$$0 = \frac{\gamma p}{\alpha} - \delta y. \tag{14}$$

If it is assumed that the glucose and insulin spaces are equal and constant, the insulin infusion rate in unit/hour/kilogram may be converted to unit/hour/liter by dividing by 0.25 (102). From the previous discussion and the results of

infusing 0.05 unit/hour/kilogram of insulin, the following substitutions into equation (14) may be made:

$\alpha$ = 0.8 hour$^{-1}$

$\beta$ = 5.0 grams/hour/unit

p = 0.2 unit/hour/liter

y = 0.14 gram/liter

to give $\delta$ = 4.4 hour$^{-1}$.

This estimate is slightly higher than the values of 3.04, 2.52, and 3.20 hour$^{-1}$ reported by Bolie (102). This increase in the estimate of delta is not accounted for by assuming that at this dosage of insulin the pancreas is releasing insulin into the blood, since this assumption would tend to increase the value of delta.

Equation (8) represents the steady state conditions during an infusion of insulin. Substitution of the previous estimates for the coefficients ( $\alpha$ , $\gamma$ , $\delta$ ) and use of the change in the steady state level of blood glucose during the infusion of 0.05 unit/hour/kilogram gave an estimate for the value of beta:

$\alpha$ = 0.8 hour$^{-1}$

$\gamma$ = 5.0 grams/hour/unit

$\delta$ = 4.4 hour$^{-1}$

p = 0.2 unit/hour/liter

$y$ = 0.28 gram/liter

therefore, from equation (8), $\beta$ = 0.202 unit/hour/gram.

In a similar manner substitution of the insulin infusion rate of 0.025 unit/hour/kilogram, gave an estimate of 0.260 unit/hour/gram for the value of beta. These estimates for beta compare favorably with the estimates of beta by Bolie. These estimates were 0.198, 0.125, and 0.300 unit/hour/gram.

However, when the values obtained with the two glucose infusion rates were used in a similar manner, the values for the estimate of beta were 0.65 and 1.13 unit/hour/gram.

Bolie (102) observed that experimental data show that normal homeostatic regulation of blood glucose exhibits physiological coefficients which approximate the critical damping of servomechanism theory. Under these criteria,

$$( \delta - \alpha )^2 = 4 \qquad (15)$$

and the maximum deviation from the normal blood glucose level after an injection of insulin is

$$y_m = \gamma \frac{D_i t_m}{Ve} \qquad (16)$$

where $t_m$ is the time after injection of insulin at which the maximum depression in blood sugar occurs, which may be shown to be

$$t_m = \frac{2}{( \delta + \alpha )}. \qquad (17)$$

The factor $D_i$ in equation (16) is the dose of insulin in unit/kilogram, V is the dilution space (0.250 liter/kilogram), and e = 2.718. Furthermore, under the criteria of critical damping the time ($t_o$) when the blood glucose returns to normal after an injection of glucose is

$$t_o = \frac{2}{(\delta - \alpha)}, \tag{18}$$

according to Bolie.

The effect on blood glucose of injecting a dose of 0.1 unit/kilogram into 3 dogs fasted 24 hours and anesthetized with pentobarbital sodium are shown in figure 21. From figure 21 the following sets of values may be obtained: $y_m$ = 0.36 gram/liter, $t_m$ = 0.5 hour; $y_m$ = 0.32 gram/liter, $t_m$ = 0.625 hour; and $y_m$ = 0.29 gram/liter, $t_m$ = 0.75 hour.

By substituting these values into equation (16) an estimate of gamma was obtained. By using 0.8 $hour^{-1}$ as an estimate of alpha and the values of $t_m$ shown above, an estimate of delta was obtained for each animal by use of equation (17). Likewise, the substitution of the values of gamma and delta from each animal into equation (15) and using 0.8 $hour^{-1}$ as a value of alpha, an estimate of beta was obtained. The 3 sets of estimates, one from each dog, were: $\beta$ = 0.294 unit/hour/gram, $\gamma$ = 4.89 grams/hour/unit, $\delta$ = 3.2 $hour^{-1}$; $\beta$ = 0.184 unit/hour/gram, $\gamma$ = 3.48 grams/hour/unit, $\delta$ = 2.4 $hour^{-1}$; and $\beta$ = 0.107 unit/hour/gram,

$\gamma$ = 2.63 grams/hour/unit, $\delta$ = 1.86 hour$^{-1}$. The mean estimates for the 3 coefficients on these dogs are: $\beta$ = 0.195 unit/hour/gram, $\gamma$ = 3.67 grams/hour/unit, $\delta$ = 2.48 hour$^{-1}$.

Figure 22 shows the effect of injecting 0.1 gram/kilogram glucose on the blood sugar level of 3 dogs which had been fasted for 24 hours and were under pentobarbital anesthesia. The blood sugar returns to normal in 50 minutes after the injection of glucose. The application of this value and 0.8 hour$^{-1}$ as a value for alpha in equation (18) gives another estimate for delta, $\delta$ = 3.2 hour$^{-1}$.

Figure 23 shows the effect on blood glucose of injecting 0.25 gram/kilogram of glucose into 3 dogs which were fasted for 24 hours and under pentobarbital anesthesia. The blood sugar returned to normal in 75 minutes after the injection of the glucose. Application of this value and substitution of $\alpha$ = 0.8 hour$^{-1}$ gives another estimate of delta, $\delta$ = 1.43 hour$^{-1}$.

By using the mean time of maximum depression of blood glucose in dogs from figure 22 in equation (17) and by using the time taken for the blood glucose to return to normal after an injection of 0.1 gram/kilogram glucose in equation (18), two equations,

$$\delta + \alpha = 3.3$$
$$\delta - \alpha = 2.4,$$

relating alpha and delta are formed. Solution of these equations gives $\delta$ = 2.85 hour$^{-1}$ and $\alpha$ = 0.45 hour$^{-1}$.

When a higher dose of glucose is injected, the time of the return to normal is 75 minutes, and

$$\delta - \alpha = 1.3$$
$$\delta + \alpha = 3.3$$

gives the following estimates $\delta$ = 4.60 hour$^{-1}$ and $\alpha$ = 1.00 hour$^{-1}$.

The mean of the last 2 estimates gives $\alpha$ = 0.73 hour$^{-1}$ and $\delta$ = 3.73 hour$^{-1}$. By using the previous estimates of the coefficients ( $\alpha$, $\gamma$ , and $\delta$ ) an estimate of $\beta$ = 0.196 unit/hour/gram may be obtained by application of equation (15).

The range of the values for the estimates of the coefficients made by Bolie (102) include the mean of most of the various estimates of the values of these coefficients obtained in this study. These estimates are shown in table 5 and confirm the reliability of the coefficients and, thus, the model itself, when applied to dogs anesthetized with sodium pentobarbital and fasted 24 hours prior to experimental use.

Limitations of the model

In most of the generalized concepts of carbohydrate homeostatic regulation it has been postulated that epinephrine plays an important role. This theory stems from the

early findings of Cannon et al. (62). They presented evidence indicating that when the blood sugar drops to low levels (40 milligrams per cent) epinephrine is released from the adrenal medulla. Furthermore, Rogoff and Nixon (158) showed that at very high levels of glucose in the blood the output of epinephrine from the adrenal glands is decreased.

The model previously discussed makes no provision for any interrelationship between the blood glucose concentration and the output of epinephrine. According to the work of De Bodo and Altszuler (63), this is correct within the ranges of blood glucose concentration observed in the experimental animals of the present investigation. De Bodo and Altszuler reported that within the physiological ranges 140 to 50 milligrams per cent in dogs, decreases in the blood glucose concentration have no effect on epinephrine blood levels. Duner (159) has shown that there is no change in the epinephrine and norepinephrine plasma levels in cats when the blood glucose level rises to 145 milligrams per cent.

In the present studies no increase in the blood pressure of 3 dogs anesthetized with pentobarbital sodium and atropinized was observed after an injection of 0.1 unit/kilogram of insulin. The blood sugar in these animals was lowered to between 60 and 50 milligrams per cent as shown in figure 21. This preparation is most sensitive to epinephrine and is used for the bioassay of epinephrine and epinephrine-like

compounds (160). In the unatropinized dog, which is less sensitive, an injection of $10^{-6}$ gram/kilogram of epinephrine causes a 10 millimeters of mercury rise in blood pressure (161). The response of the blood glucose shown in figure 21 differs from the response seen in the unanesthetized animal (162). The return to normal in the pentobarbital anesthetized animals is considerably slower, and the maximum depression occurs later than in the unanesthetized dog.

However, when dogs were treated in a similar manner except that an infusion of epinephrine sufficient to maintain a 10 millimeters of mercury increase in blood pressure was begun at 45 minutes after the injection of insulin, the blood sugar levels promptly returned to normal. These results are shown in figure 24. The amount of epinephrine infusion into each dog varied from $10^{-8}$ to $10^{-7}$ gram/minute/kilogram.

Many anesthetics are reported to have severe effects on blood glucose concentration. These effects are mediated by stimulation of the release of epinephrine. In the course of the present study 3 dogs were anesthetized with pentobarbital sodium and infused with isotonic saline at a rate of 22 milliliters/hour for periods of up to 3 hours. Little change in the blood glucose levels was observed. The maximum variation was 7 milligrams per cent from the starting level, and the mean deviation was 2.8 milligrams per cent. This is in keeping with the well-established fact that the blood glucose

levels in animals anesthetized with pentobarbital is maintained at a constant level (163, 164, 165). In addition to this, pentobarbital inhibits changes in blood glucose caused by other agents (166).

Rogoff and Nixon (158) suggested that pentobarbital may inhibit the release of epinephrine. It is well established that pentobarbital anesthesia does not abolish the release of epinephrine completely, but the rate of release is reduced (167, 168).

In view of the preceding discussion, it is felt that caution should be used in applying this model and the coefficients derived from the fasted, pentobarbital anesthetized dog to other preparations. More specifically, unanesthetized dogs, dogs anesthetized with other drugs, and, of course, other species may have different response characteristics. Furthermore, this single-compartment model does not include the effects of the renal threshold of glucose, or severe depressions of blood sugar which might increase the epinephrine release.

## MULTI-COMPARTMENT MODEL

The previous model, while adequate in some respects, does not deal specifically with the individual organs and tissues which affect the normal homeostatic blood glucose regulation. By imposing certain restrictions and making some assumptions, this model may be expanded to cover the homeostatic regulation of glucose in several of the more important compartments of the body. The presentation here will be limited to development of the extended theory as based on elaboration of the concepts previously discussed. A thorough confirmation of this extended theory involves a greatly expanded array of simultaneous measurements which are beyond the scope of this investigation, but suggest several new areas for future research.

In the multi-compartment model to be discussed the variation in blood glucose was limited to the range 50 milligrams per cent to 145 milligrams per cent. This tended to eliminate any interplay between blood glucose concentration and epinephrine release as previously discussed. Furthermore, the renal excretion of glucose was not a factor within the range of blood glucose concentrations. In addition, an attempt was made to use data obtained from dogs, although it was necessary in some instances to use, with adjustment, data from other species.

Certain assumptions were necessary to develop the model. First, it was assumed that there is no pooling of blood in any organ; that is, the input blood flow rate equals the output blood flow rate. Secondly, it was assumed that there is a rapid equilibration of glucose and insulin between the tissues and the perfusing blood, so that the concentrations of glucose and insulin in the tissue extracellular fluid and in the efferent venous blood are essentially equal. Next, it was assumed that the rate of gluconeogenesis is relatively constant during the time interval under consideration. Also, it was necessary to assume that there is no absorption of glucose by the intestine during the time under consideration. Additionally, it was assumed that there are no changes in the rate of glucagon release or that the release of glucagon serves merely to reduce the effective insulin concentration. The basis of the above limitations and assumptions has been previously discussed (101).

Also, it was assumed that the hepatic artery blood supply contributes to the liver only the metabolic needs of that organ, that is, that the portion of the insulin in the blood which is extracted by the liver via the hepatic artery is small, and the glucose in this blood supply of the liver provides all of the glucose used in the catabolic process of the liver. Some justification of this assumption was found. Markowitz et al. (169) have shown that ligation of the

hepatic artery in dogs is followed by death due to liver necrosis, while ligation of the portal system is not. This is presumably because of a lack of oxygen in the portal blood supply; but certainly if the arterial supply is sufficient for the oxygen need, it should also be adequate for the metabolic demands of normal functioning liver cells. Jamison et al. (170) have confirmed this contention through anastomosis of the vena cava and portal system. Death did not occur in the dogs, although the size of the liver was reduced, perhaps because of the loss of a supply of material to be stored.

The definition and the units assigned to the symbols used in the development of the extended model are shown in table 6. If the effect of insulin binding by the peripheral tissue can either be neglected or can be accounted for in the assumed volume of the extracellular fluid dilution space, then the rate of increase of total insulin ($V_b I_b$) in this space can be equated to the difference between the input transport rate ($F_b I_a$) and the output transport rate ($F_b I_b$). Hence, the insulin concentration of the extracellular fluid of the peripheral tissue, which includes as part of the total blood flow that flowing through the hepatic artery, is governed approximately by the equation:

$$\frac{d}{dT}(V_b I_b) = F_b I_a - F_b I_b$$

which may be written as:

$$\left(\frac{V_b}{F_b}\right)\frac{dI_b}{dT} + I_b = I_a. \tag{1}$$

The glucose concentration in the extracellular fluid of the peripheral tissue may be represented by the equation:

$$\left(\frac{V_b}{F_b}\right)\frac{dG_b}{dT} + G_b = G_a - \frac{M_b}{F_b}. \tag{2}$$

Similarly, the insulin and glucose concentrations in the blood leaving the pancreas may be represented by the following pair of equations:

$$\left(\frac{V_p}{F_p}\right)\frac{dI_p}{dT} + I_p = I_a + \frac{R}{F_p} \tag{3}$$

$$\left(\frac{V_p}{F_p}\right)\frac{dG_p}{dT} + G_p = G_a - \frac{M_p}{F_p}. \tag{4}$$

The concentrations of insulin and glucose in the blood leaving the splanchnic tissues are approximately represented by the following equations:

$$\left(\frac{V_s}{F_s}\right)\frac{dI_s}{dT} + I_s = I_a \tag{5}$$

$$\left(\frac{V_s}{F_s}\right)\frac{dG_s}{dT} + G_s = G_a - \frac{M_s}{F_s}. \tag{6}$$

The splanchnic tissues in this model are defined as the visceral or the conventional splanchnic tissues minus the pancreas.

The concentrations of insulin and glucose in the blood leaving the liver may be assumed to vary in accord with the equations:

$$\left(\frac{V_b}{F_l}\right)\frac{dI_l}{dT} + I_l = I_w - \frac{D}{F_l} \tag{7}$$

$$\left(\frac{V_l}{F_l}\right)\frac{dG_l}{dT} + G_l = G_w - \frac{H}{F_l}. \tag{8}$$

The blood flow rate in these equations is actually the total flow in the hepatic portal vein, since the hepatic artery flow is considered in this model to be part of the flow in the peripheral tissue. These primary first-order differential equations are summarized in table 6.

A schematic diagram of the simplification of the circulatory system as discussed above is shown in figure 25. The total blood flow rate is equal to the rate of flow of blood through the liver plus the flow rate through the other tissues of the body. Under the previous assumptions the flow through the hepatic artery is included in the flow through the rest of the body. The rate of blood flow through the liver, actually the blood flow rate in the portal vein, is considered to be composed of blood flowing through the

pancreas and the blood flowing through the rest of the splanchnic area. The blood flow rates may then be related by the equations:

$$F_p + F_s = F_l \tag{9}$$

$$F_l + F_b = F_t. \tag{10}$$

The transport rate of insulin (unit/hour) or the transport rate of glucose (gram/hour) is found by multiplying the blood flow rate in liter/hour times the concentration in units or grams per liter. Continuity of transport rates through the junctions shown in figure 25 thus requires that:

$$F_pI_p + F_sI_s = F_lI_w \tag{11}$$

$$F_pG_p + F_sG_s = F_lG_w \tag{12}$$

$$F_lI_l + F_bG_b = F_tG_a \tag{13}$$

$$F_lI_l + F_bI_b = F_tI_a. \tag{14}$$

Within the physiological ranges of glucose concentration the rate of insulin production by the pancreas in response to an increase in circulating glucose concentration may be approximated as a linear relationship (59). Thus, the output of insulin by the pancreas may be represented by:

$$R = K_rG_p, \tag{15}$$

where $K_r$ is a coefficient of proportionality.

The primary source of insulin metabolism is the liver, which accounts for better than 95 per cent of the insulin destruction in the body (157). The assumption that the insulin is uniformly distributed throughout the extracellular fluid of the liver, and assuming the destruction of insulin is a first-order chemical reaction, gives the equation:

$$D = K_h I_l, \tag{16}$$

where $K_h$ is the coefficient which relates the destruction of insulin to the amount of insulin leaving the liver.

The liver also serves to store glucose. The following equation:

$$H = H^o + K_l (G_l - G_o) + K_l' (I_l - I_o) \tag{17}$$

indicates that the rate of glucose storage is approximated by the sum of a basal storage rate ($H^o$), plus a term proportional to the elevation of hepatic venous glucose concentration above selected level ($G_o$), plus another term proportional to the elevation of hepatic venous insulin concentration above a selected level ($I_o$).

Within the restricted ranges of physiological variations, the rates ($M_b$, $M_s$, and $M_p$) of metabolism or entrance into the cells of glucose in the three compartments considered may be approximated by the following equations:

$$M_b = M_b^o + K_b(G_b - G_1) + K_b'(I_b - I_1) \quad (18)$$

$$M_s = M_s^o + K_s(G_s - G_2) + K_s'(I_s - I_2) \quad (19)$$

$$M_p = M_p^o + K_p(G_p - G_3) + K_p'(I_p - I_3) \quad (20)$$

where $M_b^o$, $M_s^o$, and $M_p^o$ represent rates of metabolism which are modified by the disappearance of glucose into the cells under the influence of increased glucose concentration outside of the cell and also a change in the insulin concentration. The secondary equations (9-20) are shown in table 8.

The dilution spaces can be estimated by multiplying the organ weight by a dilution space for glucose and insulin and assuming these dilution spaces remain relatively constant and are equal. A value of 0.25 liter/kilogram appears to be approximately correct for this value (102). The following values from the ratio of organ weight (gram) to body weight (100 gram) have been reported: liver 2.94, stomach and intestine 4.83, and pancreas 0.14 (171, pp. 162-163).

Assuming a body weight of 20 kilograms for the dog, the following values for the dilution spaces were calculated: $V_b$ = 4.504 liters, $V_1$ = 0.148 liter, $V_s$ = 0.240 liter, $V_p$ = 0.018 liter.

The total blood flow rate or the cardiac output for a 20 kilogram dog is about 2.5 liters/minute (172, pp. 77-80). About 0.35 liter/minute flows through the hepatic portal vein

(173). The blood flows through the pancreas at a rate of 7 milliliters/minute or 0.007 liter/minute (in dogs weighing 20 kilograms) (59). Thus, the following values were calculated: $F_p$ = 0.42 liter/hour, $F_s$ = 20.58 liters/hour, $F_l$ = 21.0 liters/hour, $F_b$ = 129.0 liters/hour, and $F_t$ = 150 liters/hour.

From the work of Metz (59) the rate (R) of insulin production by the pancreas may be estimated to range from 0.24 unit/hour to 1.20 units/hour in the 20 kilogram dog within the blood glucose ranges of this model. Also from his data, assuming that the reported value of 0.16 milliunit/milliliter is correct for the normal resting steady state insulin blood concentration, $K_r$ was estimated to have an approximate value of 0.3 (unit/hour)/(gram/liter).

Weisberg et al. (157) have calculated that the liver is capable of inactivating about 0.04 to 0.02 unit of insulin per kilogram of body weight per hour. The application of this data to the present model, assuming a 20 kilogram dog, gives an estimate of from 0.4 to 0.8 unit/hour for the maximum rate (D) of destruction of insulin by the liver. In accord with the previous discussion $K_h$ was calculated to have an approximate value of 1.5 (unit/hour)/(unit/liter).

The estimation of values for the parameters in equation (17) must be based on refinements of present experimental technique. As previously discussed, there is considerable

controversy as to whether or not insulin has a direct effect on the hepatic glucose output. Assuming insulin does not affect liver function, equation (17) may be simplified to: $H = H^{o} + K_1 (G_1 - G_0)$.

Several authors have reported that there is hepatic glucose production at arterial plasma concentrations greater than 150 milligrams per cent; hence, the rate of glucose storage (H) will be a negative quantity in the range of blood glucose concentrations for which this model was designed (174, 175, 176).

Mahler et al. (39) have reported that the glucose production rate of the liver within normal levels of blood glucose concentrations is between 0.12 to 0.35 gram of glucose per kilogram of body weight per hour. Conversion of this data to the appropriate units and application of it to the 20 kilogram dog gives -2.4 to -7.0 grams/hour for an estimate of the range of the hepatic glucose storage rate. On the basis of the previous assumption of negligible insulin effect, it may be inferred from the data presented by Landau et al. (174) that when $G_0$ equals about 0.5 gram/liter, then $H^{o}$ is equal to -7.0 grams/hour. Hence, $K_1$ may be estimated to have an approximate value of 6.3 (grams/hour)/(gram/liter). As a precaution, it should be noted that the data of Landau et al. indicates that the rate of output of carbohydrates by the liver is reduced in dogs fed high protein diets.

The rate of metabolism or glucose utilization of the tissues comprising the various compartments under consideration have not been evaluated separately. However, in the fasted, resting dog it seems reasonable to assume that the rate of metabolism of each compartment considered in figure 25 is approximately the same per gram of tissue.

Soskin and Levine (23) indicated that in the depancreatized dog the glucose utilization rate is only about 100 milligrams of glucose per kilogram of body weight per hour when the blood glucose concentration is about 50 milligrams per cent. In this preparation there is about a 10 per cent increase in the glucose utilization rate at a blood glucose concentration of 150 milligrams per cent. According to Soskin and Levine, in the intact animal the blood glucose utilization rate rises from about 215 milligrams/kilogram/hour at a blood glucose level of 50 milligrams per cent to 280 milligrams/kilogram/hour at a blood glucose level of 150 milligrams per cent.

Metz (59) found that at a blood glucose concentration of 0.80 gram/liter the blood insulin concentration was about 0.16 unit/liter. The value of 0.8 gram/liter was assigned to $G_1$, $G_2$, and $G_3$; and the value of 0.16 unit/liter was assigned to $I_1$, $I_2$, and $I_3$ (equations 18-20). From the data of Soskin and Levine the glucose utilization rate at a blood glucose concentration of 0.8 gram/liter for each compartment was

estimated, giving: $M_b^o$ = 4.2863 grams/hour; $M_s^o$ = 0.2174 gram/hour; and $M_p^o$ = 0.0063 gram/hour. Also, the difference between the glucose utilization rate at a blood glucose concentration of 0.8 gram/liter of the intact and depancreatized dog was divided by the difference between the blood insulin concentrations (0.16 unit/liter) in the two types of preparations and compartmentalized to give: $K_b'$ = 14.8272 (grams/hour)/(unit/liter); $K_s'$ = 0.7156 (gram/hour)/(unit/liter); and $K_p'$ = 0.0218 (gram/hour)/(unit/liter). Then, using the data obtained from depancreatized dogs, the increase in glucose utilization rate caused by an increase in blood glucose concentration was compartmentalized; and the following estimates resulted: $K_b$ = 0.09503 (gram/hour)/(gram/liter); $K_s$ = 0.00483 (gram/hour)/(gram/liter); and $K_p$ = 0.00014 (gram/hour)/(gram/liter).

The values for the coefficients ($K_b$, $K_s$, and $K_p$) change at blood glucose concentrations greater than 200 milligrams per cent. Also, it should be pointed out that the increase in metabolic activity in any compartment is followed by an increased basal glucose utilization ($M^o$) of that area. For example, when the animal is fed, the stomach and intestine will increase their basal rate of glucose metabolism or utilization ($M_s^o$). The finding of Dulin and Clark (177) that exercise of one limb causes an increase in the peripheral utilization of glucose in that limb, but not in the

contralateral limb, is in keeping with the above theory.

The estimated values for the various parameters derived above are summarized in table 9. These estimates, although based on data from various sources, seem to fit together quite well. For example, by using these estimates, the self-regulatory nature of glucose homeostasis is confirmed by the observation that at lower blood glucose concentrations the glucose output from the liver exceeds the total glucose utilization rate, thus tending to return the blood sugar concentrations toward normal.

One important result of this multi-compartment model of the glucose regulatory mechanism is that it can serve as a basis for designing new and more refined experiments for eliciting detailed metabolic responses of specific organs. For example, this theory shows that the influence of insulin on the rate of glucose uptake by the liver cannot be evaluated properly without controlling or measuring the effects exerted by the other organs on the levels of insulin and glucose in the hepatic circulation. Future refinements of this model might well consider renal excretion of glucose, absorption of glucose from the digestive tract, and the effects of epinephrine.

# SUMMARY AND CONCLUSIONS

Various factors affecting the *in vitro* rate of glucose uptake of rat adipose tissue were investigated. Variation between rats appeared to be a difficulty inherent in the use of this tissue for studying carbohydrate metabolism. The body weight of the rat was shown to be inversely related to the rate of uptake of glucose by the rat adipose tissue. Neither fasting of the source rat nor choline administration seems to have much effect on the rate of glucose uptake of this tissue. The treatment of rats with a ganglionic blocking agent prior to use of the tissue was found to increase the rate of glucose uptake of the tissue *in vitro* in the presence of insulin. This increase appears to be related to the decrease in the levels of epinephrine in the blood of rats pretreated with either hexamethonium or tetraethyl ammonium.

The four coefficients of a one-compartment normalized model of blood glucose regulation, consisting of a pair of first-order differential equations, were evaluated in dogs fasted 24 hours and anesthetized with sodium pentobarbital. Three sets of values for these coefficients were obtained. One set was derived from the results of infusion of glucose or insulin at constant rates over a period of several hours with one of the coefficients fixed by previous experiments.

Another set of coefficients was derived from the results of injecting glucose or insulin with one of the coefficients fixed by previous experiments. The third set of coefficients was determined from the results of injection of insulin or glucose independently of any prior studies. Results were presented which indicate that the release of epinephrine normally caused by hypoglycemia is inhibited in the fasted, pentobarbital anesthetized dog.

A multi-compartment model consisting of 8 first-order differential equations was formulated to simulate a more refined model of the blood glucose homeostatic mechanism. This model also contained 12 algebraic equations which supplement the primary differential equations. Preliminary estimates of the parameters in the extended model were presented and discussed.

## REFERENCES


1. Bernard, C. Influence de la section des Pédoncules cérébelleux moyens sur la composition de l'urine. Compt. Rend. Soc. de Biol. 1: 14-15. 1849.

2. von Mering, J. and Minkowski, O. Diabetes mellitus nach Pankreasextirpation. Arch. f. exper. Path. u. Pharmakol. 26: 371-387. 1889.

3. Laguesse, M. E. Sur la formation des ilots de Langerhans dans le pancréas. Compt. Rend. Soc. de Biol. 45: 819-820. 1893.

4. Hedan, M. E. Greffe sous-cutanée du pancréas. Compt. Rend. Soc. de Biol. 44: 307-308. 1892.

5. Macleod, J. J. R. The sugar of the blood. Physiol. Rev. 1: 208-238. 1921.

6. Banting, F. C. and Best, C. H. The internal secretion of the pancreas. J. Lab. Clin. Med. 7: 251-265. 1922.

7. de Meyer, J. Action de la secretion interne au pancréas sur differents organes. Arch. Fisiol. 7: 96-99. 1909.

8. Meyer, V. C. Chemical changes in the blood and their clinical significance. Physiol. Rev. 4: 274-328. 1924.

9. Macleod, J. J. R. Insulin. Physiol. Rev. 4: 21-68. 1924.

10. Stammers, A. D. A review of recent advances in the study of blood sugar and diabetes. Physiol. Rev. 6: 630-682. 1926.

11. Abel, J. J., Geiling, E. M. K., Rouiller, C. A., Bell, F. K., and Wintersteiner, O. Crystalline insulin. J. Pharmacol. Exper. Therap. 31: 65-85. 1927.

12. Vincent, S. Current views on "internal secretion". Physiol. Rev. 7: 288-319. 1927.

13. Nash, T. P. Phlorhizin diabetes. Physiol. Rev. 7: 385-430. 1927.

14. Cannon, W. B. Organization for physiological homeostatis. Physiol. Rev. 9: 399-431. 1929.

15. Cori, C. F. Mammalian carbohydrate metabolism. Physiol. Rev. 11: 143-275. 1931.

16. Marshall, E. K. The comparative physiology of the kidney in relation to theories of renal secretion. Physiol. Rev. 14: 133-159. 1934.

17. Jensen, H. and Evans, E. A. The chemistry of insulin. Physiol. Rev. 14: 188-209. 1934.

18. Deuel, H. J. The intermediary metabolism of fructose and galactose. Physiol. Rev. 16: 173-215. 1936.

19. Houssay, B. A. What we have learned from the toad concerning hypophyseal function. New Eng. J. Med. 214: 913-926. 1936.

20. Houssay, B. A. The hypophysis and metabolism. New Eng. J. Med. 214: 961-971. 1936.

21. Houssay, B. A. Carbohydrate metabolism. New Eng. J. Med. 214: 971-986. 1936.

22. Houssay, B. A. Certain relations between the parathyroids, the hypophysis and the pancreas. New Eng. J. Med. 214: 1128-1136. 1936.

23. Soskin, S. and Levine, R. A relationship between the blood sugar level and the rate of sugar utilization affecting the theories of diabetes. Am. J. Physiol. 120: 761-770. 1937.

24. Soskin, S., Essex, H. E., Herrich, J. R., and Mann, F. C. The mechanism of regulation of blood sugar by the liver. Am. J. Physiol. 124: 558-567. 1938.

25. Russell, J. A. The relation of the anterior pituitary to carbohydrate metabolism. Physiol. Rev. 18: 1-27. 1938.

26. Chambers, W. H. Undernutrition and carbohydrate metabolism. Physiol. Rev. 18: 248-296. 1938.

27. Soskin, S. The blood sugar: its origin, regulation and utilization. Physiol. Rev. 21: 140-193. 1941.

28. Haist, R. E. Factors affecting the insulin content of the pancreas. Physiol. Rev. 24: 409-444. 1944.

29. Ingle, D. J. Metabolic functions of the endocrine system. Ann. Rev. Physiol. 7: 527-566. 1945.

30. Lukens, F. D. W. Alloxan diabetes. Physiol. Rev. 28: 304-330. 1948.

31. Anderson, E. Metabolic functions of the endocrine glands. Ann. Rev. Physiol. 10: 329-364. 1948.

32. Bouckaert, J. P. and De Duve, C. The action of insulin. Physiol. Rev. 27: 39-71. 1948.

33. Lewis, J. J. Diabetes and the insulin-administration problem. Physiol. Rev. 29: 75-90. 1949.

34. Lazarow, A. Factors controlling the development and progression of diabetes. Physiol. Rev. 29: 49-74. 1949.

35. Turner, C. D. General endocrinology. 3rd ed. W. B. Saunders Co., Philadelphia, Pa. 1960.

36. De Duve, C. The hepatic action of insulin. In Ciba Foundation Colloquia on Endocrinology. Vol. 9. Internal secretion of the pancreas. pp. 203-223. Little, Brown, and Co., Boston, Mass. 1956.

37. Madison, L. L., Combes, B., Adams, R., and Strickland, W. The physiological significance of the secretion of insulin into the portal circulation. III. Evidence for a direct immediate effect of insulin on the balance of the glucose across the liver. J. Clin. Invest. 39: 507-522. 1960.

38. Levine, R. and Fritz, I. B. The relation of insulin to liver metabolism. Diabetes 5: 209-219. 1955.

39. Mahler, R., Shoemaker, W. C., and Ashmore, J. Hepatic action of insulin. Ann. N. Y. Acad. Sci. 82: 455-458. 1959.

40. Shoemaker, W. C., Mahler, R., Ashmore, J., Pugh, D. E., and Hastings, A. B. The hepatic glucose response to insulin in the unanesthetized dog. J. Biol. Chem. 234: 1631-1633. 1959.

41. Shoemaker, W. C., Carruthers, P. J., Powers, I. C., Wedel, L. J., and Elwyn, D. H. Effect of insulin on hepatic carbohydrate and fat metabolism in normal adrenalectomized and depancreatized dogs. (Abstract) Physiologist. 4: 109. 1961.

42. Von Itallie, T. B. and Bergen, S. S. Regulation of blood glucose, altered dynamics in certain diabetic-like states. Diabetes. 9: 286-291. 1960.

43. Lang, S., Goldstein, M. S., and Levine, R. Influence of the liver on the uptake of glucose by extrahepatic tissues. Am. J. Physiol. 177: 447-450. 1954.

44. Egdahl, R. H. and Goldberg, H. L. Changes in output per minute of pancreatic venous insulin-like activity following hyperglycemia in the dog. (Abstract) Fed. Proc. 19: 166. 1960.

45. Gemmil, C. L. The effect of insulin on the glycogen content of isolated muscles. Bull. Johns Hopkins Hosp. 66: 232-243. 1940.

46. Levine, R., Goldstein, M. S., Huddlestein, B., and Klein, S. P. Action of insulin on the "permeability" of cells to free hexosis, as studied by its effect on the distribution of galactose. Am. J. Physiol. 163: 70-76. 1950.

47. Butterfield, W. J. H. and Halling, H. E. Peripheral glucose metabolism in fasting subjects and diabetic patients. Clin. Sci. 18: 147-174. 1959.

48. Drury, D. R. and Wick, A. N. Mechanism of insulin action. Diabetes. 4: 203-205. 1955.

49. Ball, E. G. and Barrentt, R. J. Insulin and pinocytosis. Diabetes. 9: 70-71. 1960.

50. Park, C. R. and Morgan, H. E. Glucose transport and phosphorylation in muscle of diabetic animals. Diabetes. 9: 250-253. 1960.

51. Gordon, G. S. The Year Book of Endocrinology, 1959-1960. 1960.

52. Hall, C., Sordahl, L. A., and Stefko, S. The effect of insulin on oxidative phosphorylation in normal and diabetic mitochondria. J. Biol. Chem. 235: 1536-1539. 1959.

53. Cori, C. F. Enzymatic reactions in carbohydrate metabolism. Harvey Lect. 41: 253-272. 1945.

54. Zwelling, E. Insulin hypoglycemia in chick embryos. Proc. Soc. Exper. Biol. Med. 67: 192-196. 1948.

55. MacGrath, W. B. and Snedecor, J. The effect of insulin on the glucagon content of the pancreas. Diabetes. 2: 443-447. 1953.

56. Foa, P. P. The control of the secretatory activity of the islet of Langerhans. In Ciba Foundation Colloquia on Endocrinology. Vol. 9. Internal secretion of the pancreas. pp. 55-68. Little, Brown, and Co., Boston, Mass. 1956.

57. Houssay, B. A. Diabetes as a disturbance of endocrine regulation. Am. J. Med. Sci. 193: 581-606. 1935.

58. Pozza, G., Galansino, G., Hoffeld, H., and Foa, P. P. Stimulation of insulin output by monosaccharides and monosaccharide derivatives. Am. J. Physiol. 192: 497-500. 1958.

59. Metz, R. The effect of blood glucose concentration on insulin output. Diabetes. 9: 89-93. 1960.

60. Lang, C. N. H. The endocrine control of blood sugar. Diabetes. 1: 3-11. 1952.

61. Walker, G. Endocrine functions of the pancreas and regulation of blood sugar. In Campbell, E. J. M., Dickinson, C. J., and Platt, R., eds. Clinical physiology. pp. 394-396. Blackwell Scientific Publication, Oxford, England. 1960.

62. Cannon, W. B., McIver, M. A., and Bliss, S. W. Studies on the condition of activity in endocrine glands. XII. A sympathetic and adrenal mechanism in hypoglycemia. Am. J. Physiol. 69: 46-66. 1924.

63. De Bodo, R. C. and Altszuler, H. Insulin hypersensitivity and physiological insulin antagonists. Physiol. Rev. 38: 389-445. 1958.

64. Hartroft, W. S. Islets of Langerhans in man visualized by phase contrast microscopy. Proc. Am. Diabetes Assn. 10: 46-61. 1950.

65. Hartroft, W. S. Islet pathology in diabetes. Diabetes. 5: 98-104. 1955.

66. Mirsky, I. A. and Brohkahn, R. H. Inactivation of insulin by tissue extracts: I. Distribution and properties of insulin-inactivating extracts (insulinase). Arch. Biochem. 20: 1-9. 1949.

67. Mirsky, I. A. and Perisutti, G. Effect of insulinase-inhibitor on hypoglycemic action of insulin. Science. 122: 559-560. 1955.

68. Field, J. B. Observations concerning a humoral insulin antagonist during diabetic ketosis. Diabetes. 7: 433-438. 1958.

69. Haist, R. E. Islet cell function. Ann. N. Y. Acad. Sci. 82: 266-286. 1959.

70. Stadie, W. C., Haugaard, N., and Vaughn, M. The action of isotopic insulin bound to tissue. Science. 115: 486-487. 1952.

71. Haugaard, N. and Marsh, J. B. Effect of insulin on the metabolism of adipose tissue from normal rats. J. Biol. Chem. 194: 33-40. 1952.

72. Stetten, D. and Stetten, M. R. Glycogen metabolism. Physiol. Rev. 40: 505-537. 1960.

73. Vallance-Owen, J. and Wright, P. H. Assay of insulin in blood. Physiol. Rev. 40: 219-244. 1960.

74. Sanger, F. and Tuppy, H. The amino acid sequence in the phenylalanyl chain of insulin: the investigation of peptides from enzymic hydrolysates. Biochem. J. 49: 481-490. 1951.

75. Lotka, A. Elements of physical biology. Williams and Wilkins Co., Baltimore. 1925.

76. Rashevsky, N. Mathematical biophysics: physico-mathematical foundations of biology. University of Chicago Press, Chicago. 1938.

77. Wiener, N. Cybernetics. John Wiley and Sons, New York. 1943.

78. Chance, B., Greenstein, D. S. and Roughton, F. J. W. The mechanism of catalase action. I. Steady-state analysis. Arch. Biochem. Biophys. 37: 301-321. 1952.

79. Chance, B., Greenstein, D. S., Higgins, J., and Yang, C. C. The mechanism of catalase action. II. Electric analog computer studies. Arch. Biochem. Biophys. 37: 322-339. 1952.

80. Yang, C. C. An analog computor for studying biological systems. Ann. N. Y. Acad. Sci. 60: 877-883. 1955.

81. Chance, B., Higgins, J. J., and Garfinkel, D. Analogue and digital computer representation of biochemical process. Fed. Proc. 21: 75-86. 1962.

82. Grodins, F. S., Gray, J. S., Schroeder, K. R., Norins, A. L., and Jones, R. W. Respiratory responses to $CO_2$ inhalation: a theoretical study of a non-linear biological regulator. J. Appl. Physiol. 7: 283-308. 1954.

83. Dantzig, G. B., Dehaven, J. C., Cooper, I., Johnson, S. M., Deland, E. C., Kanter, H. E., and Sams, C. F. A mathematical model of the human external respiratory system. Rand Corporation (Santa Monica, Calif.) U. S. Air Force Project Rand Research Memorandum RM-2519. 1959.

84. Bellville, J. W. and Seed, J. C. Respiratory carbon dioxide response curve computer. Science 130: 1079-1083. 1959.

85. Fritts, H. W., Richards, D. W., and Cournand, A. Oxygen consumption of tissues in the human lung. Science. 133: 1070-1072. 1961.

86. Clynes, M. Respiratory control of heart rate: Laws derived from analog computor simulation. Inst. Radio Eng. Trans. Med. Elect. ME-7: 2-14. 1959.

87. Reeve, E. B., Allen, T. H., and Roberts, J. E. Blood volume regulation. Ann. Rev. Physiol. 22: 349-380. 1960.

88. Warner, H. R. Use of analogue computers in the study of control mechanisms in the circulation. Fed. Proc. 21: 87-91. 1962.

89. Hardy, J. D. Physiology of temperature regulation. Physiol. Rev. 41: 521-606. 1961.

90. Shore, M. L. Biological applications of kinetic analysis of a two-compartment open system. J. Appl. Physiol. 16: 771-782. 1961.

91. Brownell, G. I., Cavicchi, R. V., and Perry, K. E. An electrical analog of analysis of compartmental biological systems. Rev. Sci. Inst. 24: 704-710. 1953.

92. Bolie, V. W. and Cholvin, N. R. Effects of capillary permeability on tracers in the blood. Proc. Iowa Acad. Sci. 67: 507-510. 1960.

93. Wollman, S. H. Kinetics of accumulation of radioiodine by thyroid gland: longer time intervals. Am. J. Physiol. 202: 189-192. 1962.

94. Silverman, M. and Burgen, S. V. Application of analogue computer measurement on intestinal absorption rates with tracers. J. Appl. Physiol. 16: 911-913. 1961.

95. Clynes, M. Unidirectional rate sensitivity: a biocybernetic law of reflex and humoral system as physiologic channels of control and communication. Ann. N. Y. Acad. Sci. 92: 946-969. 1961.

96. Wallach, D. P. and Thomas, R. C. Psicofuranine. VIII. Some pharacological observations. Antibiot. Chemother. 9: 722-729. 1959.

97. Garrett, E. R., Thomas, R. C., Wallach, D. P., and Alway, C. D. Psicofuranine: kinetics and mechanisms in vivo with the application of the analog computer. J. Pharm. Exper. Therap. 130: 106-118. 1960.

98. Stark, L. and Cornsweet, T. N. Testing a servoanalytic hypothesis for pupil oscillations. Science. 127: 558. 1958.

99. Danzinger, L. and Elmergreen, G. L. The thyroid pituitary homeostatic mechanism. Bull. Math. Biophys. 18: 1-13. 1956.

100. Roston, S. Mathematical representation of some endocrinological systems. Bull. Math. Biophys. 21: 271-282. 1959.

101. Bolie, V. W. Glucose-insulin feedback theory. Internatl. Conf. Med. Elect. 3rd, London, 1960. Proc. 3: 39-42. 1960.

102. Bolie, V. W. Coefficients of normal blood glucose regulation. J. Appl. Physiol. 16: 783-788. 1961.

103. Ikkos, D. and Luft, R. On the volume of distribution of glucose in man. Acta Endocrinol. 25: 334-344. 1957.

104. Ikkos, D. and Luft, R. On the intravenous glucose tolerance test. Acta Endocrinol. 25: 312-334. 1957.

105. Wajchenberg, B. L., Pieroni, R. R., and Hoxter, G. Preliminary mathematical model for glucagon-induced hepatic glycogenolysis in man. Metabolism. 10: 585-588. 1961.

106. Peterson, L. H. Introduction to the principles of digital and analogue computers. Fed. Proc. 21: 69-74. 1962.

107. Jones, R. W., Green, D. G., and Pinter, R. B. Mathematical simulation of certain receptor and effector organs. Fed. Proc. 21: 97-102. 1962.

108. Bickford, R. G., Poole, E. W., and McCarthy, C. E. Some applications in EEG analysis. Fed. Proc. 21: 103-108. 1962.

109. Bellman, R. Mathematical experimentation and biological research. Fed. Proc. 21: 109-111. 1962.

110. Farley, B. G. Some results of computer simulation of neuron-like nets. Fed. Proc. 21: 92-96. 1962.

111. Keilin, D. and Hartree, E. F. Specificity of glucose oxidase. Biochem. J. 50: 331-341. 1952.

112. Froesch, R. E. and Renold, A. E. Specific enzymatic determination of glucose in blood and urine using glucose oxidase. Diabetes. 5: 1-6. 1956.

113. Beach, E. F. and Turner, J. J. An enzymatic method for glucose determination in body fluids. Clin. Chem. 4: 462-473. 1958.

114. Huggett, A. S. G. and Nixon, D. A. Use of glucose oxidase and o-diansidine in determination of blood and urinary glucose. Lancet. 273: 368-270. 1957.

115. Middleton, J. E. and Griffiths, W. J. Rapid colorimetric micromethod for estimating glucose in blood and C. S. F. using flucose oxidase. Brit. Med. J. 1957: 1525-1527. 1957.

116. Middleton, J. E. Experience with a glucose oxidase method for estimating glucose in blood and C. S. F. Brit. Med. J. 1959: 824-826. 1959.

117. Kingsley, G. R. and Getchell, G. Direct ultramicro glucose oxidase method for determination of glucose in biological fluids. Clin. Chem. 6: 466-470. 1960.

118. Saloman, L. L. and Johnson, J. E. Enzymatic microdetermination of glucose in blood and urine. Anal. Chem. 1: 453-456. 1959.

119. Worthington Biochemical Corporation. Glucostat for the enzymatic determination of glucose. The Worthington Descriptive Manual No. 11: 75-59. 1961.

120. Munck, A. Effect of cortisol on glucose uptake by rat epidymal fat pad. Endocrinol. 68: 178-179. 1961.

121. Antoniades, H. N. Hormones in human plasma. Little, Brown and Co., Boston, Mass. 1960.

122. Randle, P. J. and Taylor, K. W. Insulin. In Gray, C. H. and Bacharach, A. L., eds. Hormones in blood. pp. 11-47. Academic Press., New York. 1961.

123. Gellhorn, E., Feldman, J., and Allen, A. Assay of insulin on hypophysectomized, adrenodemedullated and hypophysectomized-adrenodemedullated rats. Endocrinol. 29: 137-140. 1941.

124. Anderson, E., Lender, E. and Sutton, G. A sensitive method for the assay of insulin in blood. Am. J. Physiol. 149: 350-354. 1947.

125. Gemmill, C. L. The effects of glucose and of insulin on the metabolism of the isolated diaphragm of the rat. Bull. John Hopkins Hosp. 68: 329-336. 1941.

126. Vallance-Owen, J. and Hulock, B. Estimation of plasma insulin by the rat diaphragm method. Lancet. 278: 68-70. 1954.

127. Stadie, W. C. and Zapp, J. A. The effect of insulin upon the synthesis of glycogen by rat diaphragm in vitro. J. Biol. Chem. 170: 55-65. 1947.

128. Beigelman, P. M. Bioassay for insulin-like activity utilizing glucose uptake by adipose tissue. Metabolism. 9: 580-586. 1960.

129. Ball, E. G., Martin, D. B., and Cooper, O. Studies on the metabolism of adipose tissue. I. The effect of insulin on glucose utilization as measured by the manometric determination of carbon dioxide output. J. Biol. Chem. 234: 774-780. 1959.

130. Renold, A. E., Martin, D. B., Dagenais, Y. M., Steinke, J., Nickerson, R. J., and Sheps, M. C. Measurement of small quantities of insulin-like activity using rat adipose tissue. I. A proposed procedure. J. Clin. Invest. 39: 1487-1498. 1960.

131. Sheps, M. C., Nickerson, R. J., Dagenais, Y. M., Steinke, J., Martin, D. B., and Renold, A. E. Measurement of small quantities of insulin-like activity using rat adipose tissue. II. Evaluation of performance. J. Clin. Invest. 39: 1499-1510. 1960.

132. Yalow, R. S. and Berson, S. A. Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39: 1157-1175. 1960.

133. Beigelman, P. M. Insulin-like activity of normal and diabetic human serum. Diabetes. 8: 29-35. 1959.

134. Beigelman, P. M. and Onoprienko, I. S. Improvement of adipose tissue insulin bioassay by lengthening incubation time. Metabolism. 10: 163-164. 1961.

135. Jeanrenaud, B. and Renold, A. E. Studies on rat adipose tissue in vitro. IV. Metabolic patterns produced in rat adipose tissue by varying insulin and glucose concentration independently from each other. J. Biol. Chem. 234: 3082-3087. 1959.

136. Winegard, A. I. and Renold, A. E. Studies on rat adipose tissue in vito. I. Effects of insulin on the metabolism of glucose, pyruvate, and acetate. J. Biol. Chem. 233: 267-272. 1958.

137. Hagen, J. H. Effect of insulin on the metabolism of adipose tissue from hyperthyroid rats. J. Biol. Chem. 235: 2600-2603. 1960.

138. Hagen, J. M., Ball, E. G., and Cooper, O. Studies on the metabolism of adipose tissue. II. The effects of changes in the ionic composition of the medium upon response to insulin. J. Biol. Chem. 234: 781-786. 1960.

139. Winegard, A. I. and Renold, A. E. Studies on rat adipose tissue in vitro. II. Effects of insulin on the metabolism of specifically labeled glucose. J. Biol. Chem. 233: 273-276. 1958.

140. Portman, O. W. and Stare, F. J. Dietary regulation of serum cholesterol levels. Physiol. Rev. 39: 407-442. 1959.

141. Olson, R. E. and Vester, J. W. Nutrition-endocrine interrelationship in the control of fat transport in man. Physiol. Rev. 40: 677-733. 1960.

142. Jeanrenaud, B. and Renold, A. E. Studies on rat adipose tissue in vitro. VII. Effects of adrenal cortical hormones. J. Biol. Chem. 235: 2217-2223. 1960.

143. Best, C. H. and Lucas, C. C. Choline: chemistry and significance as a dietary factor. Vitamins and Hormones. 1: 1-58. 1950.

144. Drill, V. A. Hepatotoxic agents: Mechanism of action and dietary interrelationship. Pharmacol. Rev. 4: 1-42. 1952.

145. Corriea, P. R. and Magalhaes, E. Effect of prior epinephrine upon adipose tissue sensitivity to insulin in vitro. Metabolism. 10: 808-811. 1961.

146. Hagen, J. H. and Ball, E. G. Studies on the metabolism of adipose tissue. IV. The effect on insulin and adrenalin on glucose utilization lactate production and net gas exchange. J. Biol. Chem. 235: 1545-1549. 1960.

147. Cori, C. F. and Cori, G. T. The mechanism of epinephrine action. I. The influence of epinephrine on the carbohydrate metabolism of fasting rats with a note on new formation of carbohydrates. J. Biol. Chem. 79: 309-319. 1928.

148. Walaas, O. and Walaas, E. Effect of epinephrine on rat diaphragm. J. Biol. Chem. 187: 769-776. 1950.

149. Eccles, R. M. Intracellular potentials recorded from a mammalian sympathetic ganglion. J. Physiol. 130: 572-584. 1955.

150. Paton, W. D. M. and Zaimis, E. J. Paralysis of autonomic ganglia by methonium salts. Brit. J. Pharmacol. 6: 155-168. 1951.

151. Burn, J. H. The relation of adrenalin to acetylcholine in the nervous system. Physiol. Rev. 25: 377-394. 1945.

152. DiSalvo, R. J., Bloom, W. L., Burst, R. W., Ferguson, R. W., and Ferris, E. B. A comparison of the metabolic and circulatory effects of epinephrine, norepinephrine, and insulin hypoglycemia with observation on the influence of autonomic blocking agents. J. Clin. Invest. 35: 568-577. 1956.

153. Monge, C. C., Corcoran, A. C., del Greco, F., and Page, I. H. Volume distribution of hexamethonium in nephrectomized dogs. Am. J. Physiol. 157: 158-167. 1949.

154. Haugaard, N., Vaughan, M., Haugaard, E. S., and Stadie, W. C. Studies of radioactive injected labeled insulin. J. Biol. Chem. 208: 549-563. 1954.

155. Greenley, P. O. The basal insulin requirement of depancreatized dogs. Am. J. Physiol. 120: 345-348. 1937.

156. Best, C. H. Carbohydrate metabolism. In Best, C. H. and Taylor, N. B., eds. The physiological basis of medical practice. 7th edition. pp. 808-838. Williams and Wilkins Co., Baltimore, Md. 1961.

157. Weisberg, H. F., Friedman, A., and Levine, R. Inactivation or removal of insulin by the liver. Am. J. Physiol. 158: 332-336. 1949.

158. Rogoff, J. M. and Nixon, E. N. Epinephrine output from the adrenal glands in experimental diabetes. Am. J. Physiol. 37: 440-445. 1937.

159. Duner, H. The influence of the blood glucose levels on the secretion of adrenaline and noradrenaline from the suprarenal gland. Acta Physiol. Scand. Supp. No. 102: 1-77. 1953.

160. The pharmacopoeia of the United States of America. 13th edition. Mack Printing Co., Easton, Pa. 1947.

161. Nickerson, M. and Goodman, L. S. Pharmacological and physiological aspects of adrenergic blockade with special reference to dibenamine. Fed. Proc. 7: 397-409. 1948.

162. DeBodo, R. C., Steele, R., Altszuler H., Dunn, A., Armstrong, D. T., and Bishop, J. S. Further studies on the mechanism of action of insulin. Metabolism. 8: 520-530. 1959.

163. Hrubetz, M. C. and Blackberg, S. N. Influence of nembutal, pentothal, seconal, amytal and phenobarbital on blood sugar concentration and carbohydrate metabolism. Am. J. Physiol. 122: 759-764. 1938.

164. Jackson, E. L. The influence of sodium pentobarbital upon the reaction of normal rabbits to successive doses of insulin. J. Pharm. Exper. Therap. 42: 277-285. 1931.

165. Olmstead, J. M. D. and Giragossintz, M. A. Some effects of amytal anesthesia. J. Lab. Clin. Med. 16: 342-361. 1931.

166. Watts, D. T. The effect of methadone isomers, morphine and pentobarbital on the blood glucose. J. Pharm. Exper. Therap. 102: 269-271. 1951.

167. Guyton, A. C. and Gillespie, W. M. Constant infusion of epinephrine and destruction in the body. Am. J. Physiol. 165: 319-327. 1951.

168. Hume, D. M. Discussion of paper by Page, I. H.: Some neurohumoral and endocrine aspects of shock. Fed. Proc. Supp. 9, 20: 87-97. 1961.

169. Markowitz, J., Rappaport, A., and Scott, A. C. The function of the hepatic artery in the dog. Am. J. Digest. Dis. 16: 344-348. 1949.

170. Jamison, W. L., Dugan, T. J., Bailey, C. P., and Ramirez, H. P. R. Experiences in experimental portacaval anastomosis. Surgery. 30: 484-499. 1951.

171. Spector, W. S., ed. Handbook of biological data. W. B. Saunders Co., Philadelphia, Pa. 1956.

172. Altman, P. L., ed. Handbook of circulation. W. B. Saunders Co., Philadelphia, Pa. 1959.

173. Grindlay, J. H. Measurement of the blood flow of the liver. Am. J. Physiol. 132: 489-498. 1941.

174. Landau, B. R., Leonards, J. R., and Barry, F. M. Regulation of blood glucose concentration: response of liver to glucose administration. Am. J. Physiol. 201: 41-46. 1961.

175. Bondy, P. K., James, D. F., and Farrar, B. W. Studies of the role of the liver in human carbohydrate metabolism by the venous cather technic. I. Normal subjects under fasting conditions and following the injection of glucose. J. Clin. Invest. 28: 238-244. 1948.

176. Cahill, G. F., Ashmore, J., Earle, A. S., and Zottu, S. Glucose penetration into the liver. Am. J. Physiol. 192: 491-496. 1958.

177. Dulin, W. E. and Clark, J. J. Studies concerning a possible humoral factor produced by working muscles its influence on glucose utilization. Diabetes. 10: 289-297. 1961.

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# TABLES AND FIGURES

Table 1. Reproducibility of glucose determination on glucose standard (100 mg. per cent), dog blood and Krebs-bicarbonate medium

| Fluid | Number of determinations | Mean±Standard deviation |
|---|---|---|
| Glucose standard | 10 | 0.6025 ± 0.0018 O.D. |
| Dog blood | 10 | 80.66 ± 00.47 mg. per cent |
| Krebs-bicarbonate medium | 10 | 1.053 ± 0.007 mg./ml. |

Table 2. Analysis of variance of glucose uptake by epididymal fat tissue between rats

| Source of variation | Degrees of freedom | Sum of squares | Mean square |
|---|---|---|---|
| Rats | 23 | 40.633 | 1.767[a] |
| Tissue | 120 | 36.028 | 0.300 |
| Total | 143 | 76.661 | |

[a]Highly significant.

Table 3. The increase by various treatment on the glucose uptake (mg./gm./hr.) of rat epididymal fat tissue

| Treatment | Number of animals | Increased glucose uptake (treated minus control) | Significance p level |
|---|---|---|---|
| Fast (8 hr.) | 12 | -0.009 ± 0.135 | < 0.05 |
| Choline injection (10 mg./kg.) | 12 | -0.085 ± 0.136 | < 0.05 |
| Hexamethonium injection (20 mg./kg.) | 18 | 0.496 ± 0.176 | > 0.05 |
| Hexamethonium injection (20 mg./kg.) no insulin in solution | 18 | 0.157 ± 0.180 | < 0.05 |
| Hexamethonium in medium (0.0325 mg./ml.) | 7 | 0.093 ± 0.141 | < 0.05 |
| Tetraethyl ammonium injection (5 mg./kg.) | 18 | 0.425 ± 0.145 | > 0.05 |

Table 4. Effect of infusion of insulin or glucose on blood glucose concentration

| Treatment | Number of animals | Blood glucose (mg. percent) during infusion (minutes after start) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 135 | 150 | 165 | 180 | 210 |
| Glucose 0.25 gm./kg./hr. | 10 | 79±9 | 93±13 | 96±12 | 93±12 | 89±12 | 82±13 | 82±7 | 83±7 | 83±6 | 81±12 | 81±12 | 81±12 | 81±13 | ---- |
| Glucose 0.5 gm./kg./hr. | 11 | 77±6 | 97±7 | 109±8 | 117±6 | 114±13 | 113±12 | 95±6 | 91±4 | 91±4 | 91±4 | 91±5 | ---- | ---- | ---- |
| Insulin 0.025 units/kg./hr. | 10 | 83±8 | 82±8 | 81±7 | 79±7 | 76±8 | 75±7 | 74±7 | 73±7 | 72±7 | 72±7 | 70±8 | 68±12 | 70±12 | ---- |
| Insulin 0.05 units/kg./hr. | 9 | 78±6 | 71±6 | 66±8 | 61±8 | 57±5 | 56±4 | 54±4 | 52±4 | 50±2 | 50±2 | 50±2 | ---- | 51±4 | 51±4 |

Table 5. Comparison of coefficients obtained from the fasted pentobarbital anesthetized dog and those obtained from a combination of other sources

| Coefficients | Range[a] | Mean of estimates by various methods | | |
|---|---|---|---|---|
| $\alpha$ | 0.625-0.916 | 0.800 | 0.800 | 0.730 |
| $\beta$ | 0.125-0.300 | 0.561 | 0.610 | 0.196 |
| $\gamma$ | 3.23 -5.00 | 5.00 | 3.67 | 3.67 |
| $\delta$ | 2.52 -3.20 | 4.40 | 2.48 | 3.73 |

[a]From Bolie (102).

Table 6. Definition of symbols of multicompartment model

| Symbol | Definition | Units |
|---|---|---|
| G | Glucose concentration | Gram/liter |
| I | Insulin concentration | Unit/liter |
| F | Blood flow rate | Liter/hour |
| V | Dilution space | Liter |
| M | Rate of metabolism of glucose | Gram/hour |
| R | Rate of release of insulin | Unit/hour |
| D | Rate of destruction of insulin | Unit/hour |
| H | Rate of storage of glucose | Gram/hour |
| T | Time | Hour |
| Subscripts: | | |
| a | Relates to arterial blood | |
| p | Relates to pancreas | |
| l | Relates to liver | |
| s | Relates to splanchnic area (less pancreas and liver) | |
| b | Relates to peripheral tissue (includes hepatic artery) | |
| w | Relates to portal vein | |
| t | Relates to total flow | |

Table 7. Fundamental equations of multicompartment model[a]

Peripheral tissue (includes hepatic artery distribution)

$$\left(\frac{V_b}{F_b}\right)\frac{dI_b}{dT} + I_b = I_a \qquad (1)$$

$$\left(\frac{V_b}{F_b}\right)\frac{dG_b}{dT} + G_b = G_a - \frac{M_b}{F_b} \qquad (2)$$

Pancreas

$$\left(\frac{V_p}{F_p}\right)\frac{dI_p}{dT} + I_p = I_a + \frac{R}{F_p} \qquad (3)$$

$$\left(\frac{V_p}{F_p}\right)\frac{dG_p}{dT} + G_p = G_a - \frac{M_p}{F_p} \qquad (4)$$

Splanchnic tissues (less pancreas and liver)

$$\left(\frac{V_s}{F_s}\right)\frac{dI_s}{dT} + I_s = I_a \qquad (5)$$

$$\left(\frac{V_s}{F_s}\right)\frac{dG_s}{dT} + G_s = G_a - \frac{M_s}{F_s} \qquad (6)$$

Liver

$$\left(\frac{V_l}{F_l}\right)\frac{dI_l}{dT} + I_l = I_w - \frac{D}{F_l} \qquad (7)$$

$$\left(\frac{V_l}{F_l}\right)\frac{dG_l}{dT} + G_l = G_w - \frac{H}{F_l} \qquad (8)$$

[a]Partially from unpublished notes of V. W. Bolie, Iowa State University, Ames, Iowa.

Table 8. Secondary equations of multicompartment model[a]

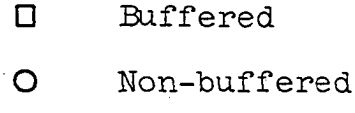
| | |
|---|---|
| Flow in portal vein | |
| $F_p + F_s = F_l$ | (9) |
| $F_pI_p + F_sI_s = F_lI_w$ | (11) |
| $F_pG_p + F_sG_s = F_lG_w$ | (12) |
| Total flow | |
| $F_l + F_b = F_t$ | (10) |
| $F_lG_l + F_bG_b = F_tG_a$ | (13) |
| $F_lI_l + F_bI_b = F_tI_a$ | (14) |
| Islet function | |
| $R = K_rG_p$ | (15) |
| Liver function | |
| $D = K_hI_l$ | (16) |
| $H = H^o + K_l(G_l - G_o) + K_l(I_l - I_o)$ | (17) |
| Metabolism | |
| $M_b = M_b^o + K_b(G_b - G_l) + K_b'(I_b - I_l)$ | (18) |
| $M_s = M_s^o + K_s(G_s - G_2) + K_s'(I_s - I_2)$ | (19) |
| $M_p = M_p^o + K_p(G_p - G_3) + K_p'(I_p - I_3)$ | (20) |

[a]Partially from unpublished notes of V. W. Bolie, Iowa State University, Ames, Iowa.

Table 9. Estimated values for various parameters of the multicompartment model

| Parameter | Estimated value | |
|---|---|---|
| $V_b$ | 4.50 | liter |
| $V_l$ | 0.148 | liter |
| $V_s$ | 0.240 | liter |
| $V_p$ | 0.018 | liter |
| $F_b$ | 129.0 | liter/hour |
| $F_l$ | 21.0 | liter/hour |
| $F_s$ | 20.6 | liter/hour |
| $F_p$ | 0.42 | liter/hour |
| $F_t$ | 150.0 | liter/hour |
| $K_r$ | 0.3 | (unit/hour)/(gram/liter) |
| $K_n$ | 1.5 | (unit/hour)/(unit/liter) |
| $H^o$ | -7.0 | gram/hour |
| $G_0$ | 0.50 | gram/liter |
| $K_l$ | 6.3 | (gram/hour)/(gram/liter) |
| $M_b^o$ | 4.29 | gram/hour |
| $G_1$ | 0.80 | gram/liter |
| $I_1$ | 0.16 | unit/liter |
| $K_b$ | 0.095 | (gram/hour)/(gram/liter) |
| $K_b'$ | 14.8 | (gram/hour)/(unit/liter) |
| $M_s^o$ | 0.217 | gram/hour |
| $G_2$ | 0.80 | gram/liter |
| $I_2$ | 0.16 | unit/liter |
| $K_s$ | 0.00483 | (gram/hour)/(gram/liter) |
| $K_s'$ | 0.751 | (gram/hour)/(unit/liter) |
| $M_p^o$ | 0.0063 | gram/hour |
| $G_3$ | 0.80 | gram/liter |
| $I_3$ | 0.16 | unit/liter |
| $K_p$ | 0.00014 | (gram/hour)/(gram/liter) |
| $K_p'$ | 0.0218 | (gram/hour)/(unit/liter) |

Figure 1. Effect of glucose concentration on optical density in buffered and non-buffered samples



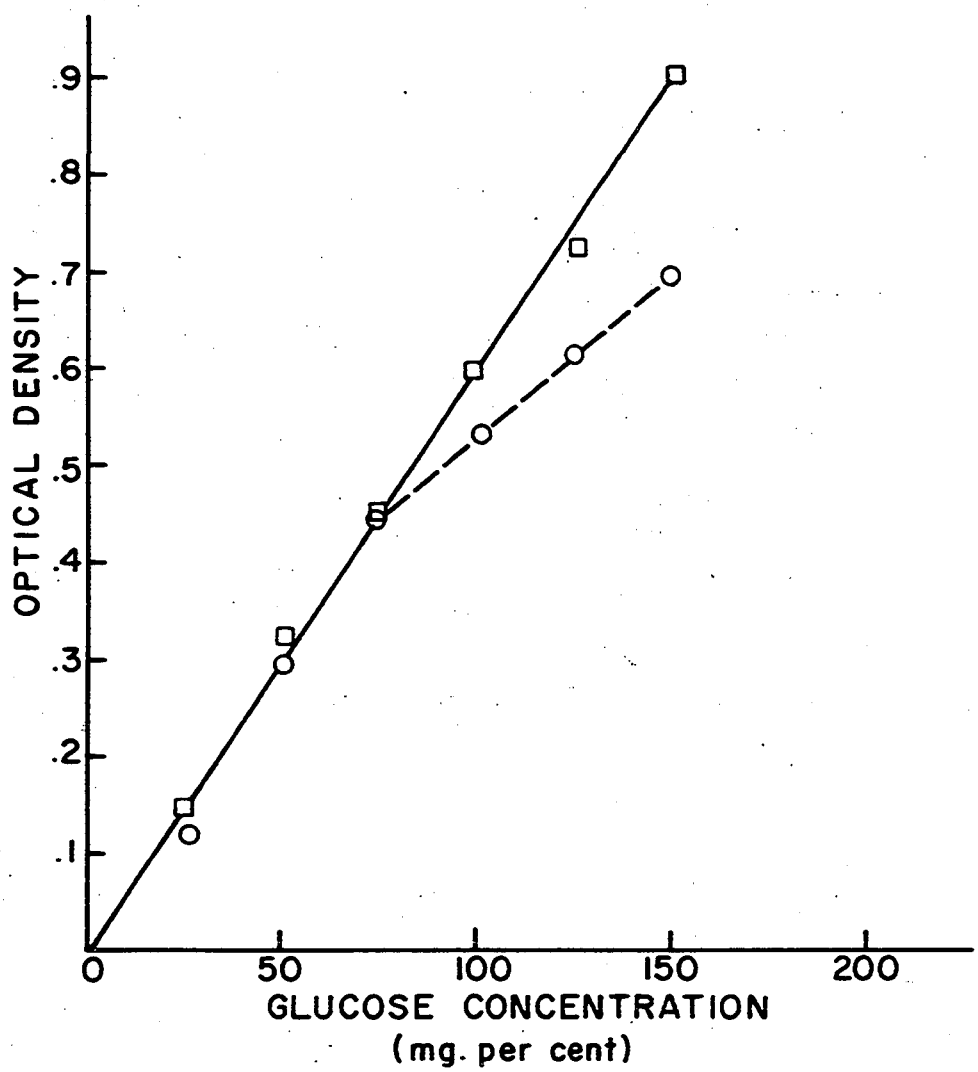

OPTICAL DENSITY
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
50
100
150
200
GLUCOSE CONCENTRATION
(mg. per cent)

Figure 2. The effect of temperature on the development of color using glucose oxidase method

□ Room temperature (22°C)

○ 37°C

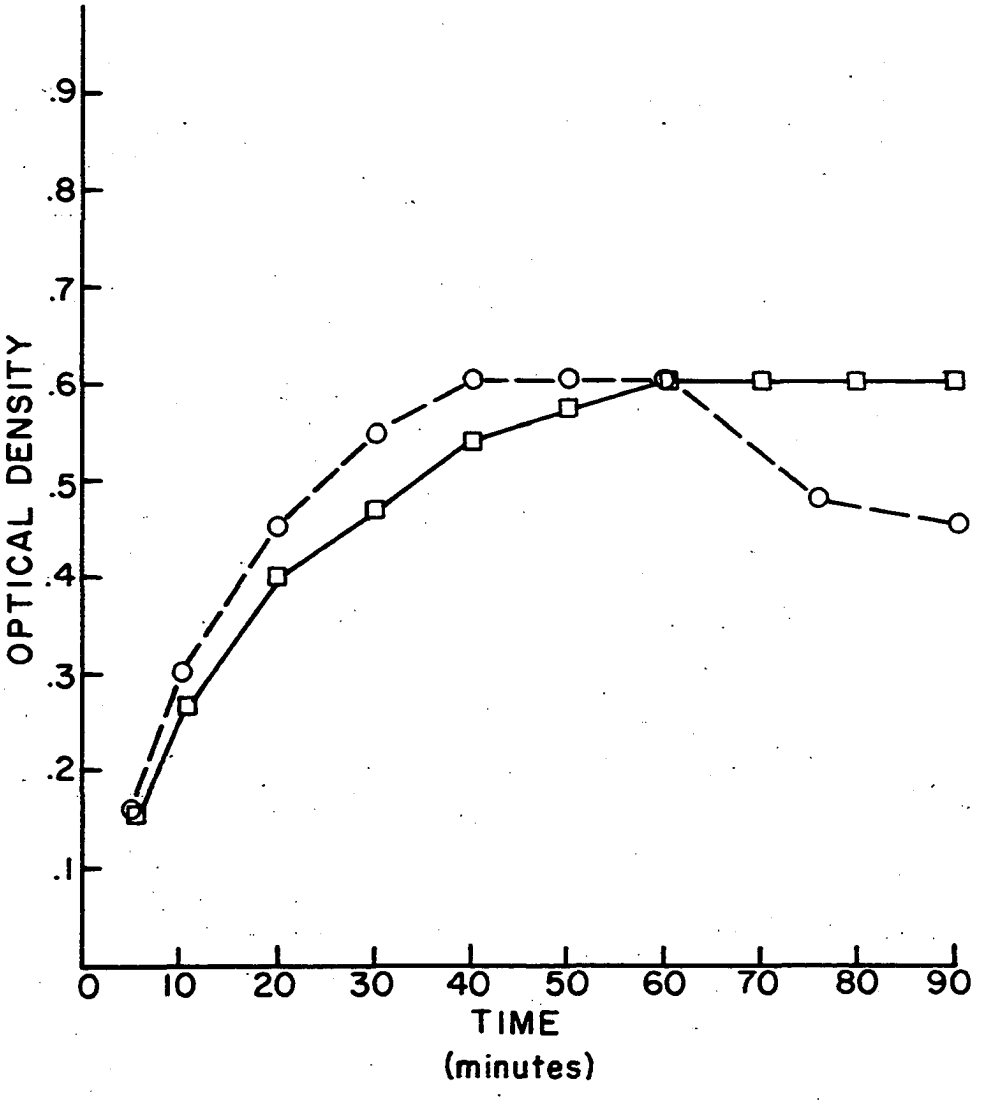

OPTICAL DENSITY
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
10
20
30
40
50
60
70
80
90
TIME
(minutes)

Figure 3. Relation of body weight to glucose uptake by epididymal adipose tissue from 46 rats

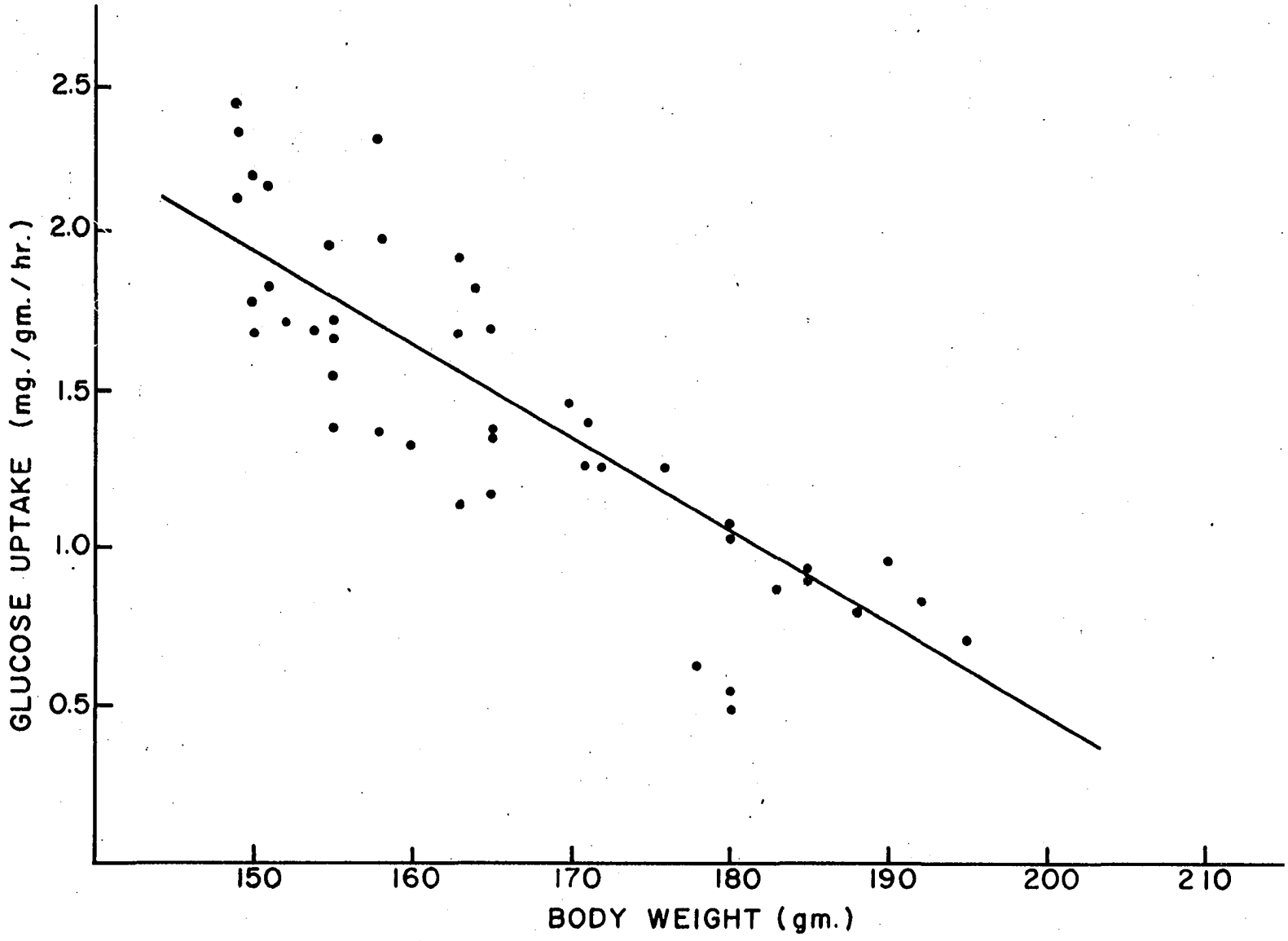
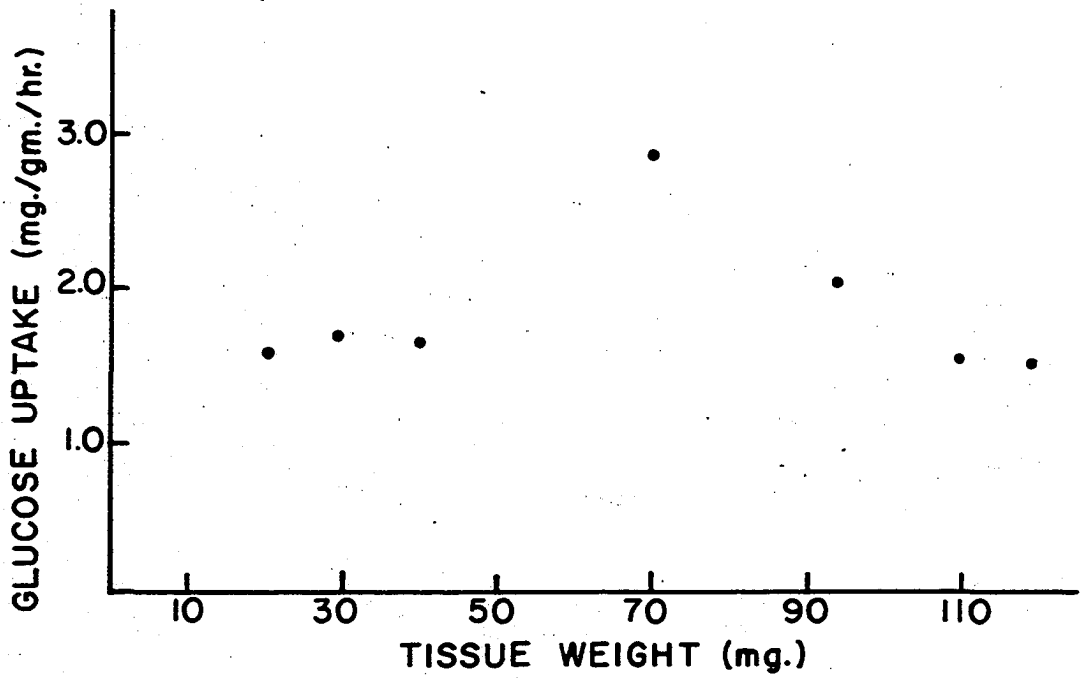

GLUCOSE UPTAKE (mg./gm./hr.)
2.5
2.0
1.5
1.0
0.5
150
160
170
180
190
200
210
BODY WEIGHT (gm.)

Figure 4. Relation of tissue weight to glucose uptake by adipose tissue samples from rat A

Figure 5. Relation of tissue weight to glucose uptake by adipose tissue samples from rat B


GLUCOSE UPTAKE (mg./gm./hr.)
3.0
2.0
1.0
10
30
50
70
90
110
TISSUE WEIGHT (mg.)

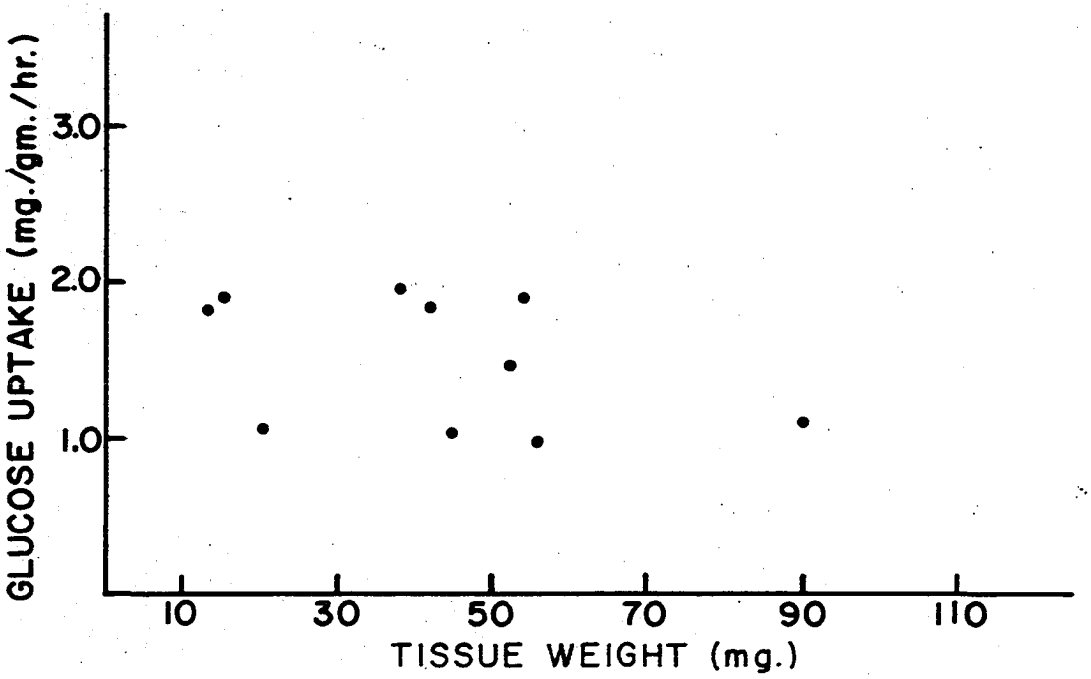
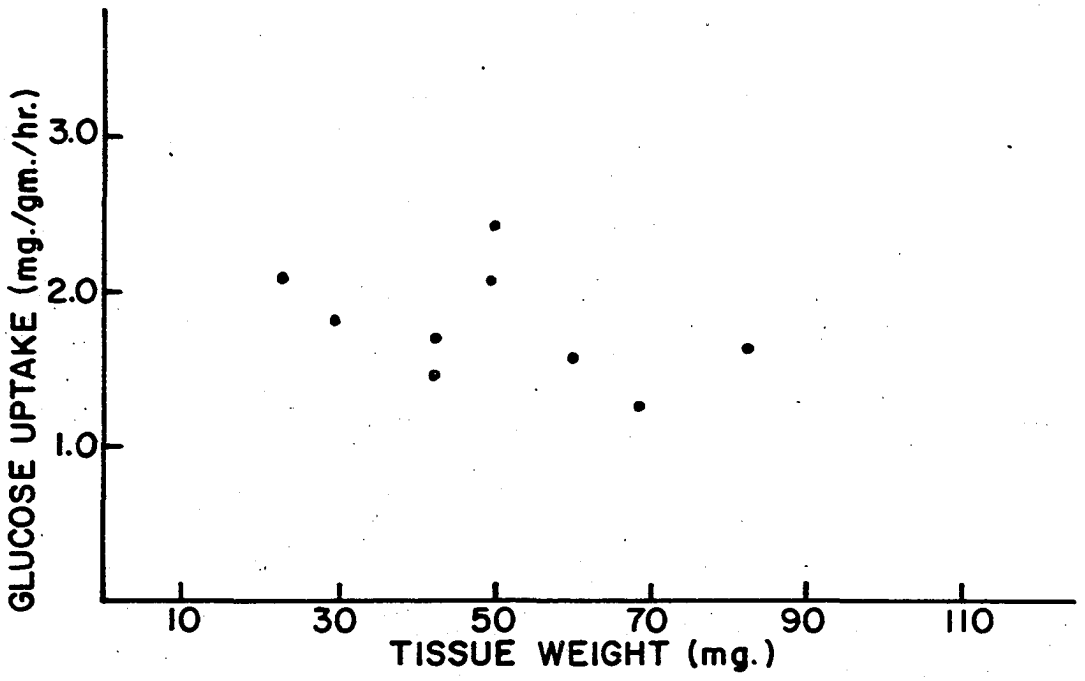

GLUCOSE UPTAKE (mg./gm./hr.)
3.0
2.0
1.0
10
30
50
70
90
110
TISSUE WEIGHT (mg.)

Figure 6. Relation of tissue weight to glucose uptake by adipose tissue sample from rat C

Figure 7. Relation of tissue weight to glucose uptake by adipose tissue sample from rat D


GLUCOSE UPTAKE (mg./gm./hr.)
3.0
2.0
1.0
10
30
50
70
90
110
TISSUE WEIGHT (mg.)

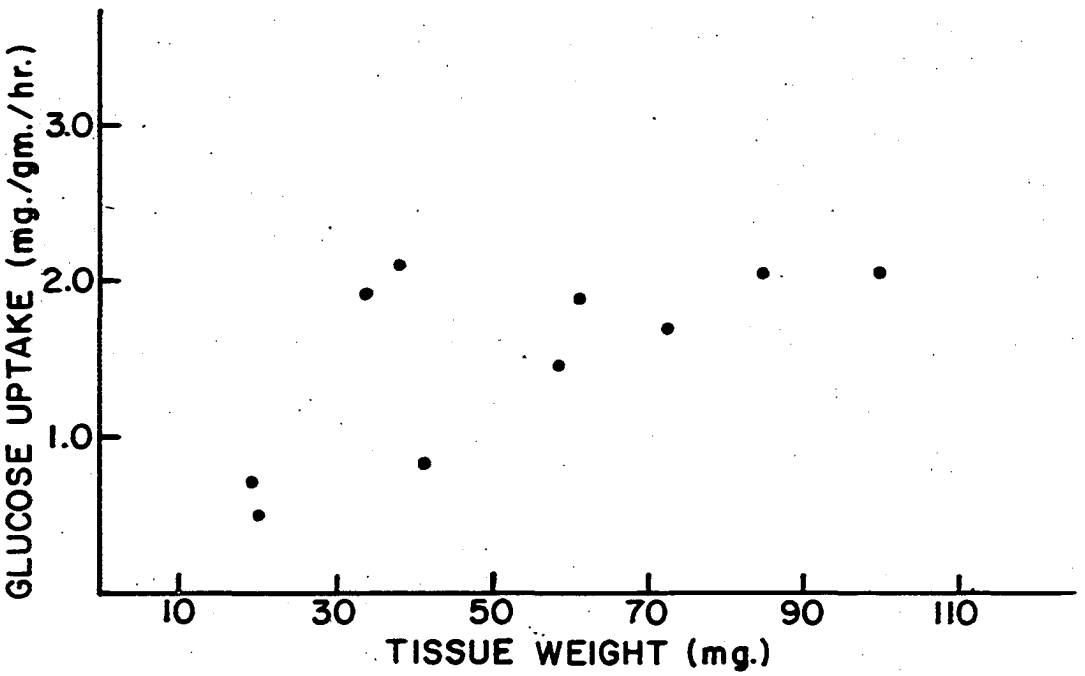
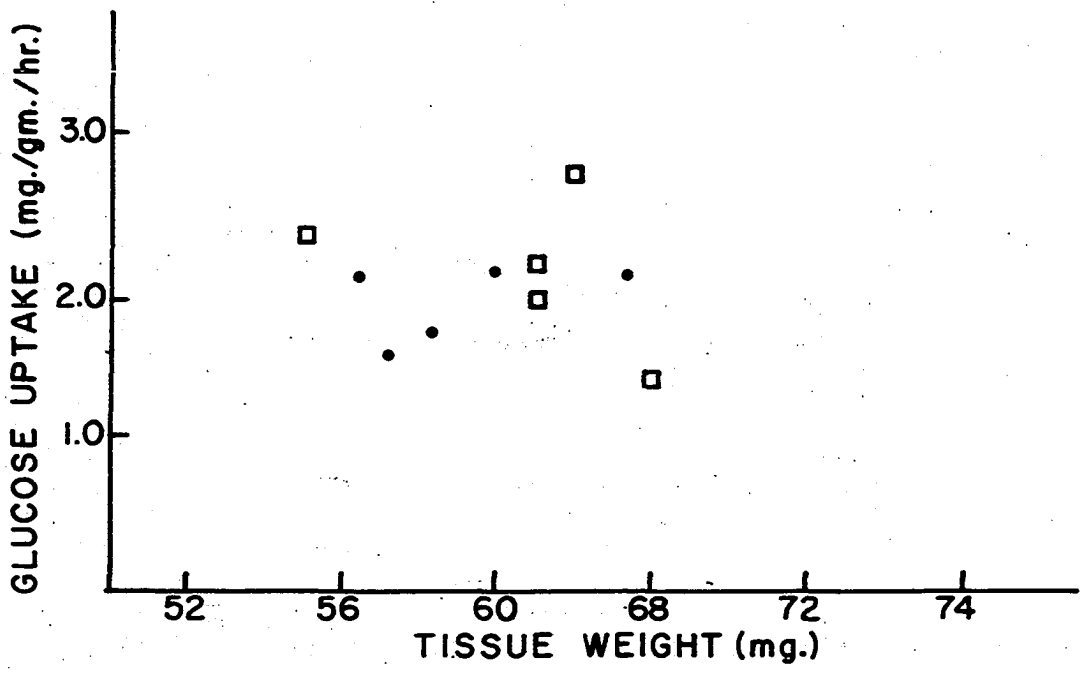

GLUCOSE UPTAKE (mg./gm./hr.)
3.0
2.0
1.0
10
30
50
70
90
110
TISSUE WEIGHT (mg.)

Figure 8. Relation of tissue weight to glucose uptake by adipose tissue samples from rat E

- ● Whole pieces
- □ Cut into many smaller pieces

Figure 9. Relation of tissue weight to glucose uptake by adipose tissue samples from rat F

- ● Whole pieces
- □ Cut into many smaller pieces


GLUCOSE UPTAKE (mg./gm./hr.)
3.0
2.0
1.0
52
56
60
68
72
74
TISSUE WEIGHT (mg.)

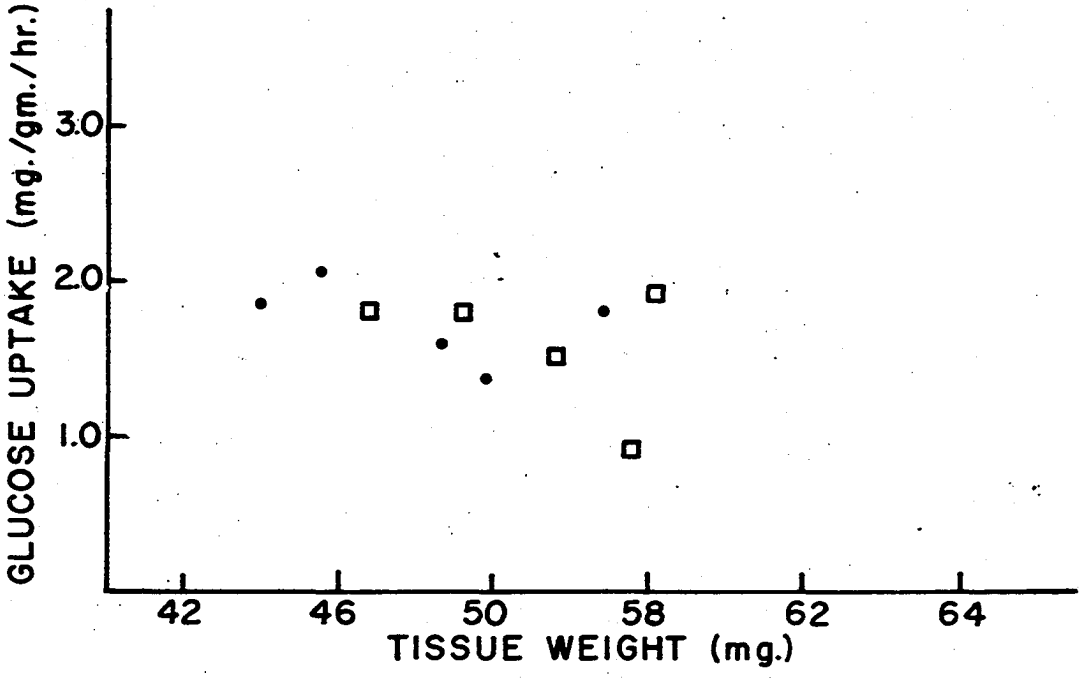
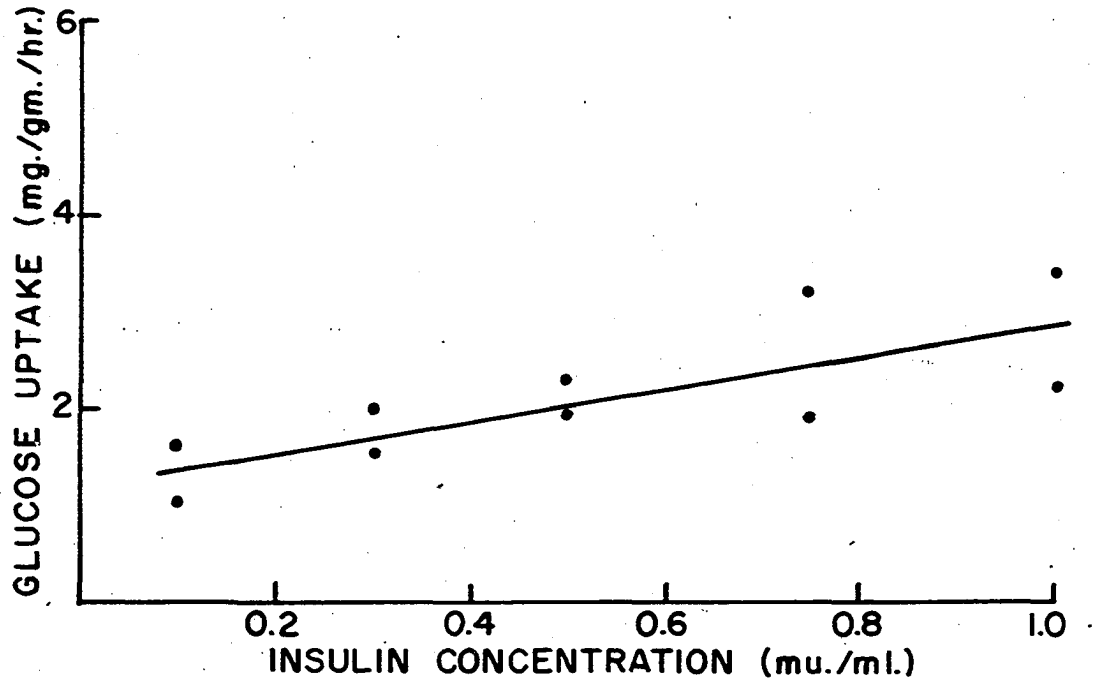

GLUCOSE UPTAKE (mg./gm./hr.)
3.0
2.0
1.0
42
46
50
58
62
64
TISSUE WEIGHT (mg.)

Figure 10. Relation of insulin concentration to glucose uptake by adipose tissue samples from rat G

Figure 11. Relation of insulin concentration to glucose uptake by adipose tissue samples from rat H


GLUCOSE UPTAKE (mg./gm./hr.)
6
4
2
0.2
0.4
0.6
0.8
1.0
INSULIN CONCENTRATION (mu./ml.)

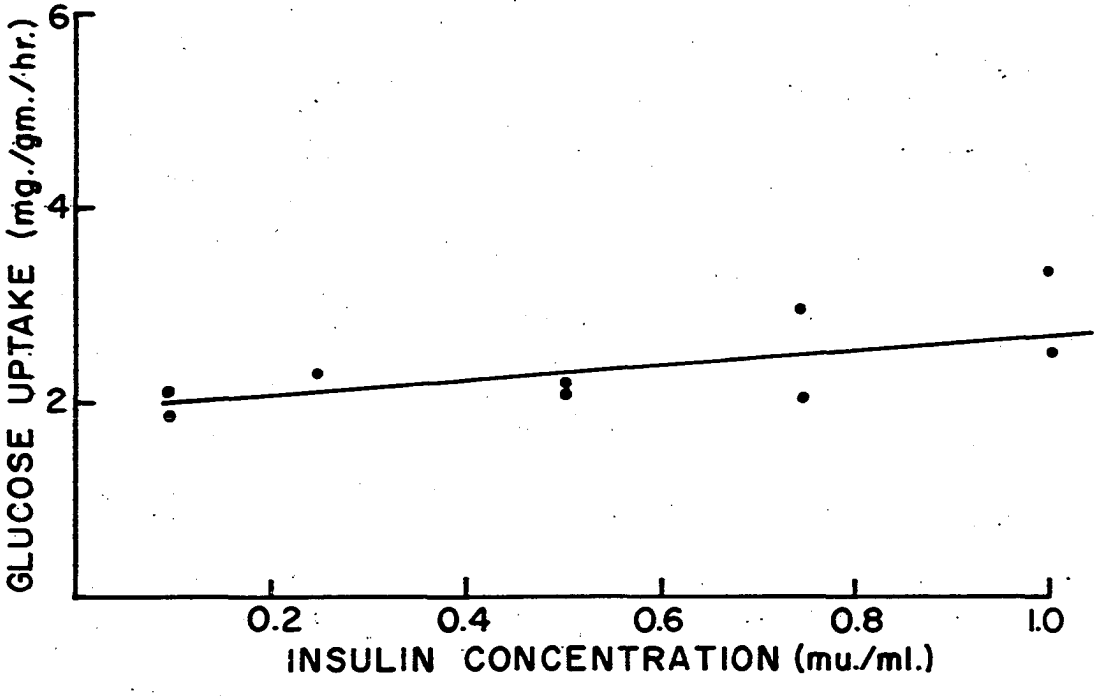
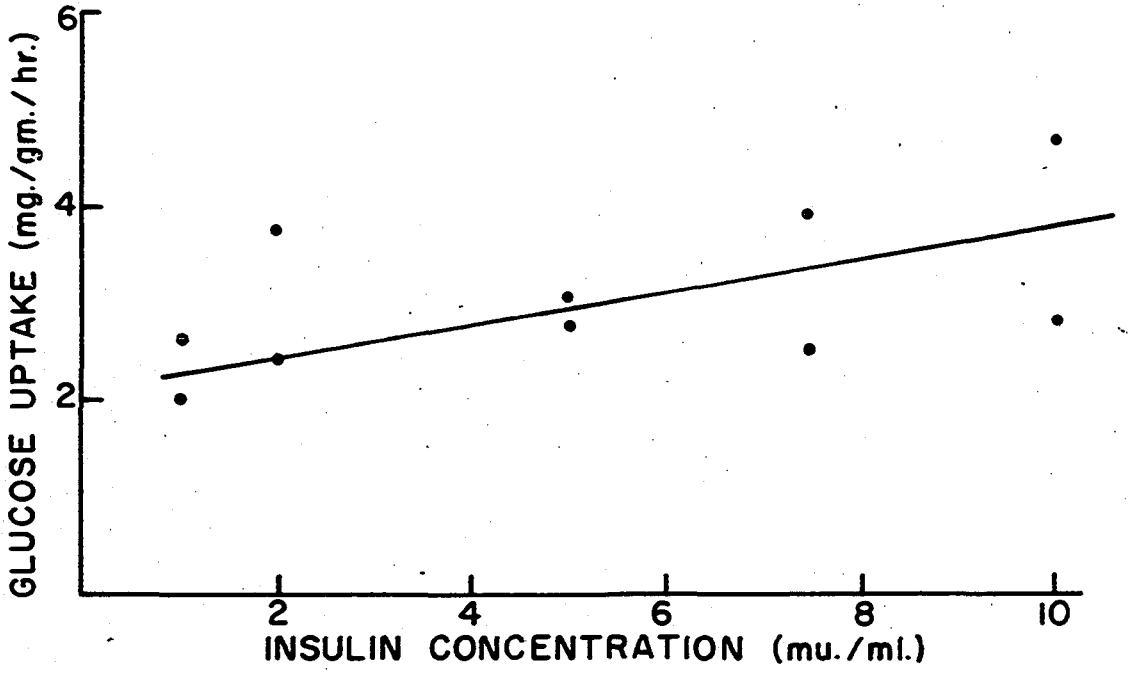

GLUCOSE UPTAKE (mg./gm./hr.)
6
4
2
0.2
0.4
0.6
0.8
1.0
INSULIN CONCENTRATION (mu./ml.)

Figure 12. Relation of insulin concentration to glucose uptake by adipose tissue samples from rat I

Figure 13. Relation of insulin concentration to glucose uptake by adipose tissue samples from rat J


GLUCOSE UPTAKE (mg./gm./hr.)
6
4
2
2
4
6
8
10
INSULIN CONCENTRATION (mu./ml.)

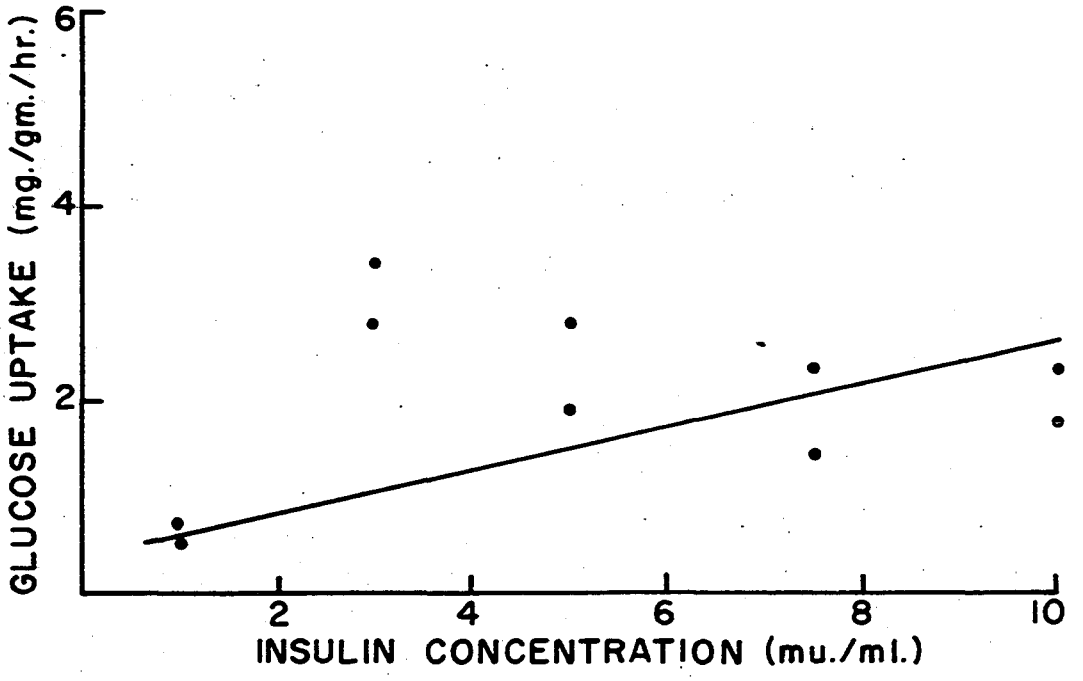

GLUCOSE UPTAKE (mg./gm./hr.)
6
4
2
2
4
6
8
10
INSULIN CONCENTRATION (mu./ml.)

Figure 14. Relation of insulin concentration to glucose uptake by adipose tissue samples from rat K

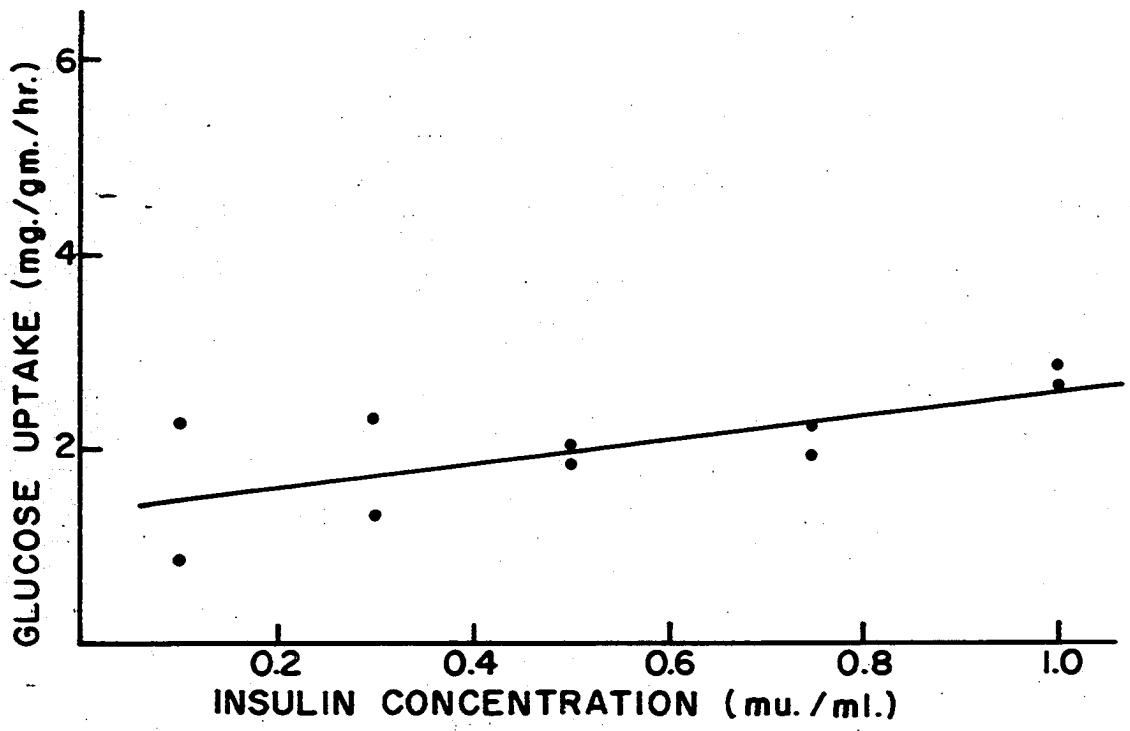

GLUCOSE UPTAKE (mg./gm./hr.)
6
4
2
0.2
0.4
0.6
0.8
1.0
INSULIN CONCENTRATION (mu./ml.)

Figure 15. Changes in mean blood glucose concentrations in 9 dogs during infusion of 0.05 unit/hour/kilogram of insulin.

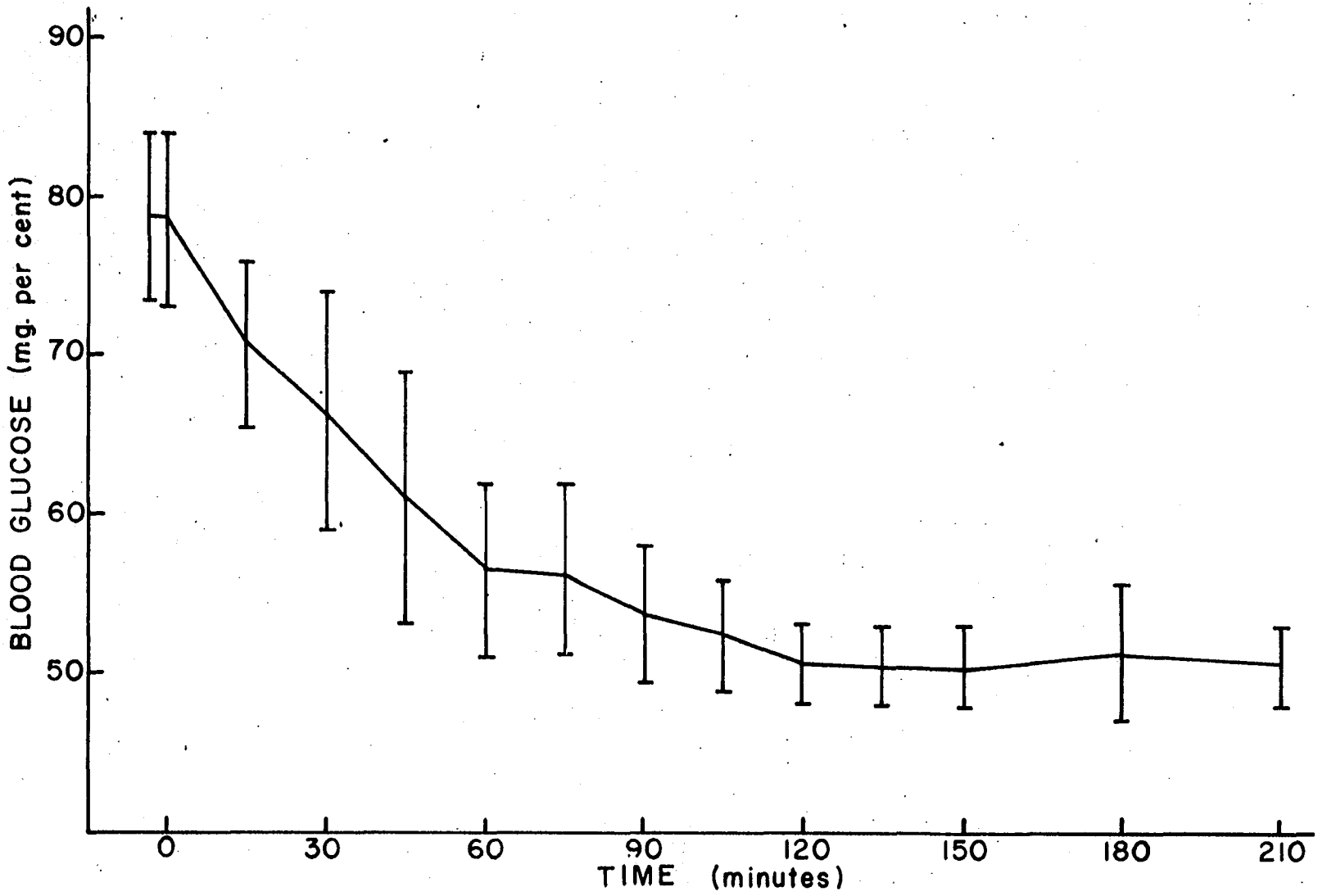

BLOOD GLUCOSE (mg. per cent)
90
80
70
60
50
0
30
60
90
120
150
180
210
TIME (minutes)

Figure 16. Changes in mean blood glucose concentrations in 10 dogs during infusion of 0.025 unit/hour/kilogram of insulin

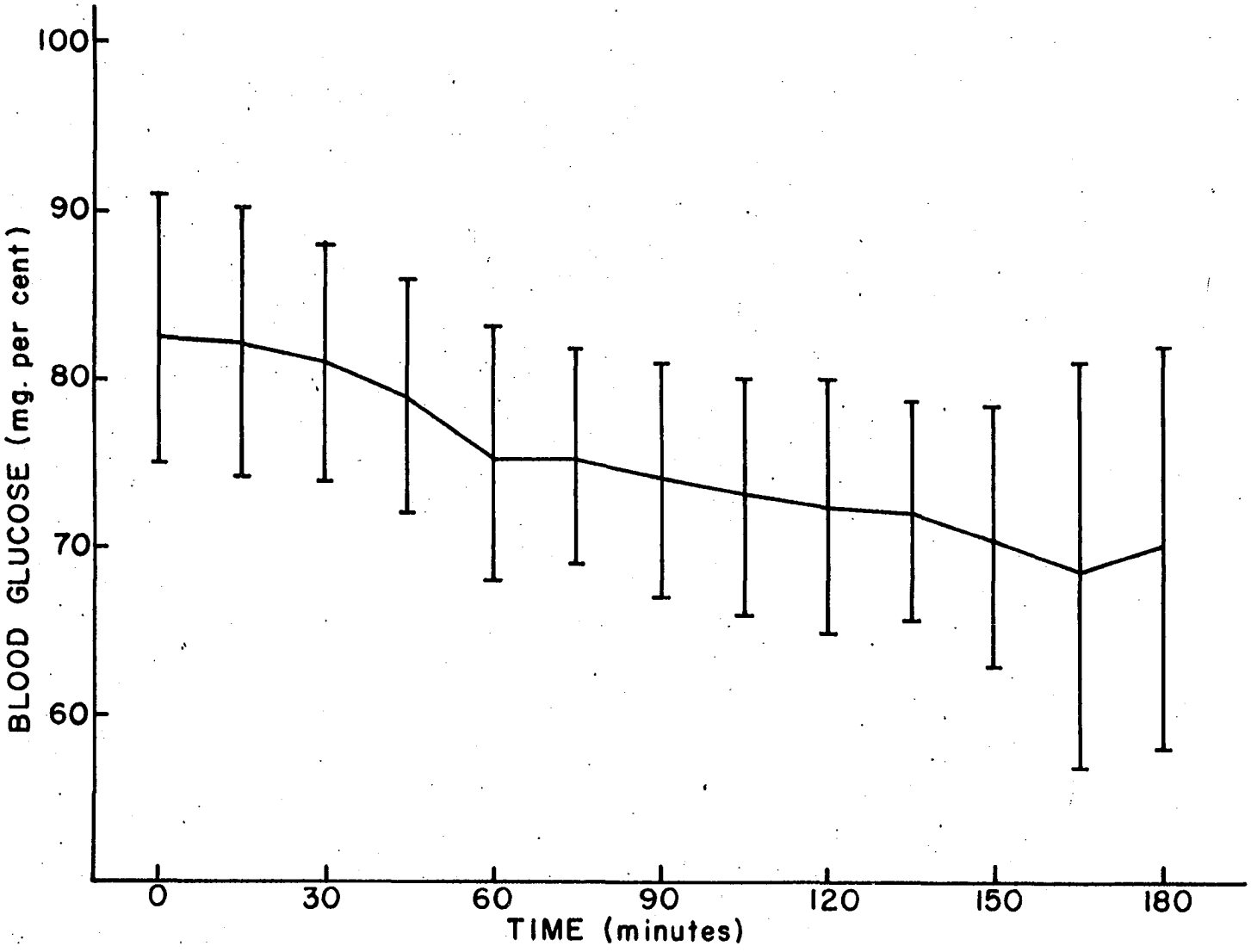

100
90
80
70
60
BLOOD GLUCOSE (mg. per cent)
0
30
60
90
120
150
180
TIME (minutes)

Figure 17. Changes in mean blood glucose concentrations in 11 dogs during infusion of 0.5 gram/hour/kilogram of glucose

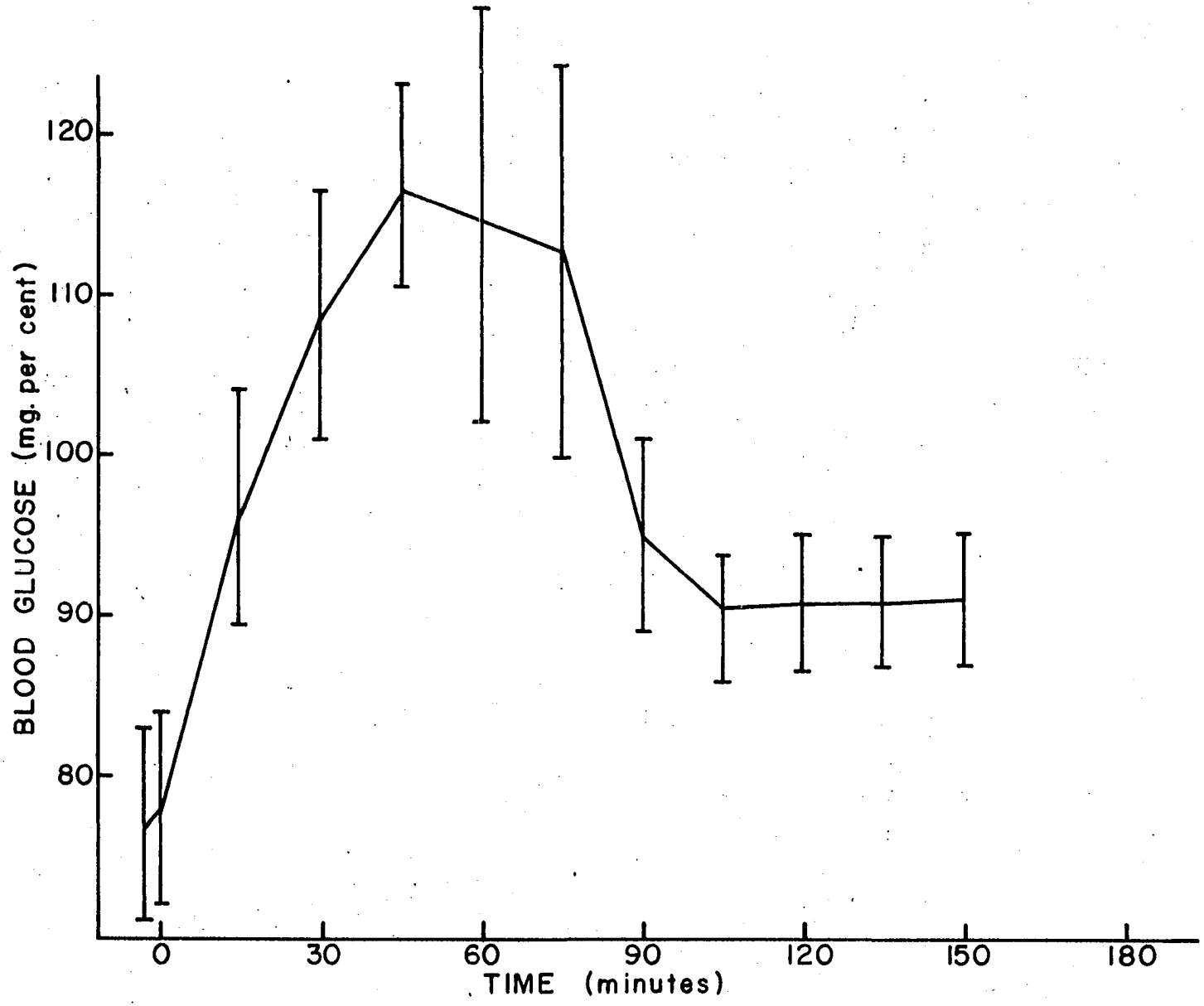

BLOOD GLUCOSE (mg. per cent)
120
110
100
90
80
0
30
60
90
120
150
180
TIME (minutes)

Figure 18. Changes in mean blood glucose concentrations in 10 dogs during infusion of 0.25 gram/hour/kilogram of glucose

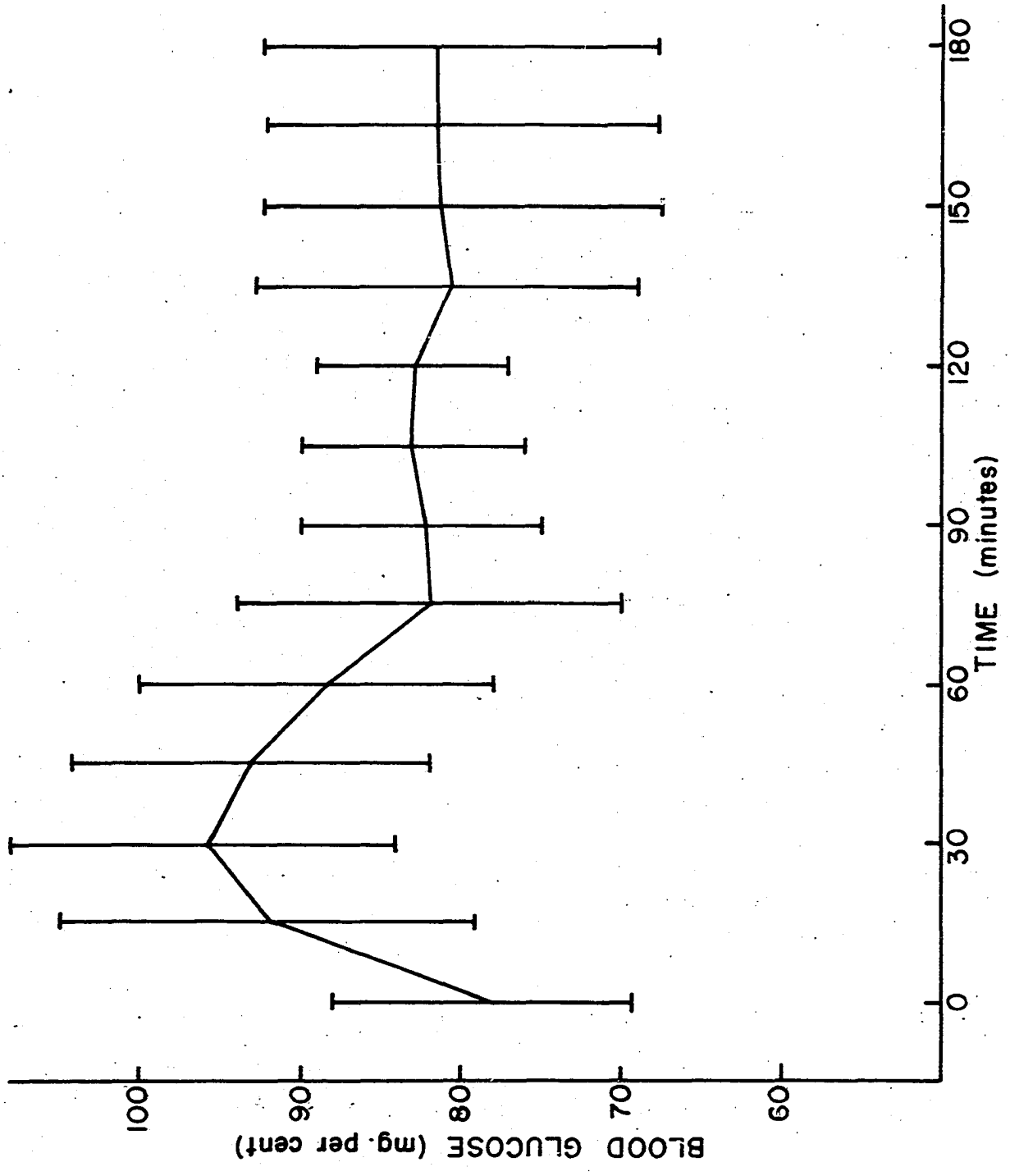
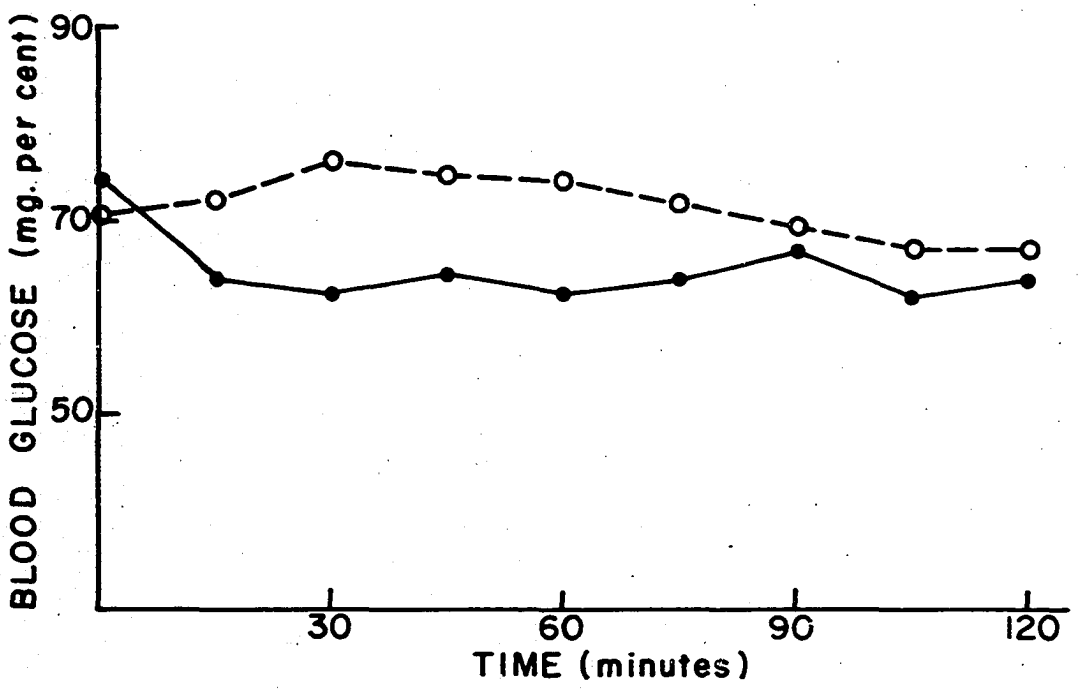

BLOOD GLUCOSE (mg. per cent)
100
90
80
70
60
0
30
60
90
120
150
180
TIME (minutes)

Figure 19. Effect of infusion of a combination of insulin (0.04 unit/hour/kilogram) and glucose (0.25 gram/hour/kilogram) on blood glucose concentration in dogs A and B

Figure 20. Effect of infusion of a combination of insulin (0.11 unit/hour/kilogram) and glucose (0.25 gram/hour/kilogram) on blood glucose concentration in dogs C and D


BLOOD GLUCOSE (mg. per cent)
90
70
50
30
60
90
120
TIME (minutes)

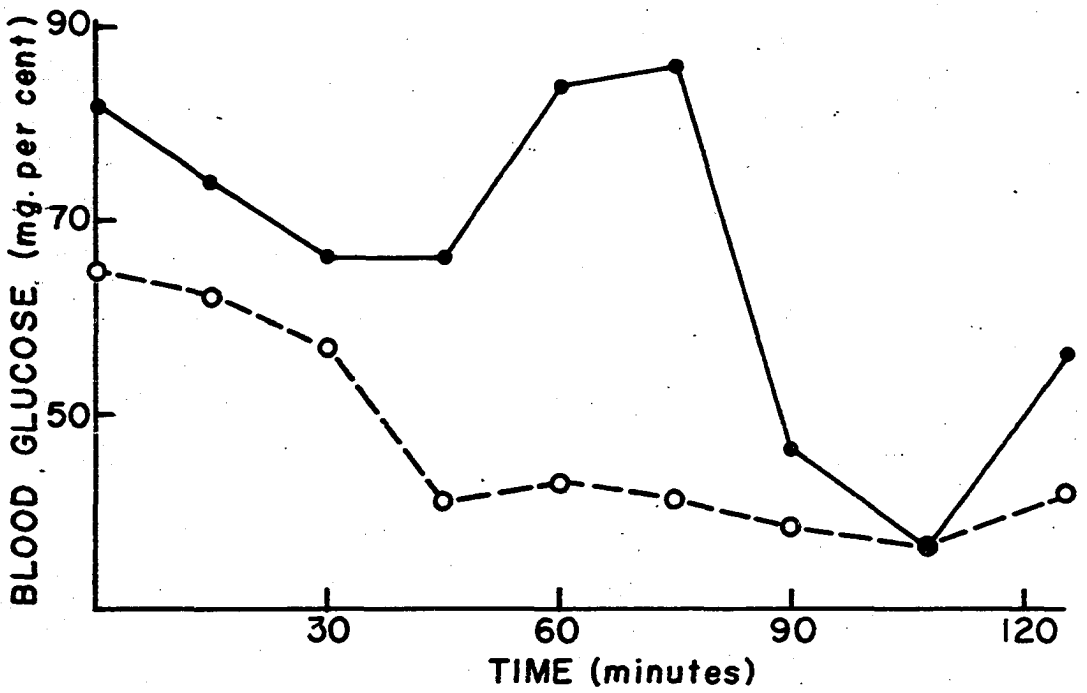

BLOOD GLUCOSE (mg. per cent)
90
70
50
30
60
90
120
TIME (minutes)

Figure 21. Changes in blood glucose concentrations in dogs (E, F, G) after injection of 0.1 unit/kilogram of insulin

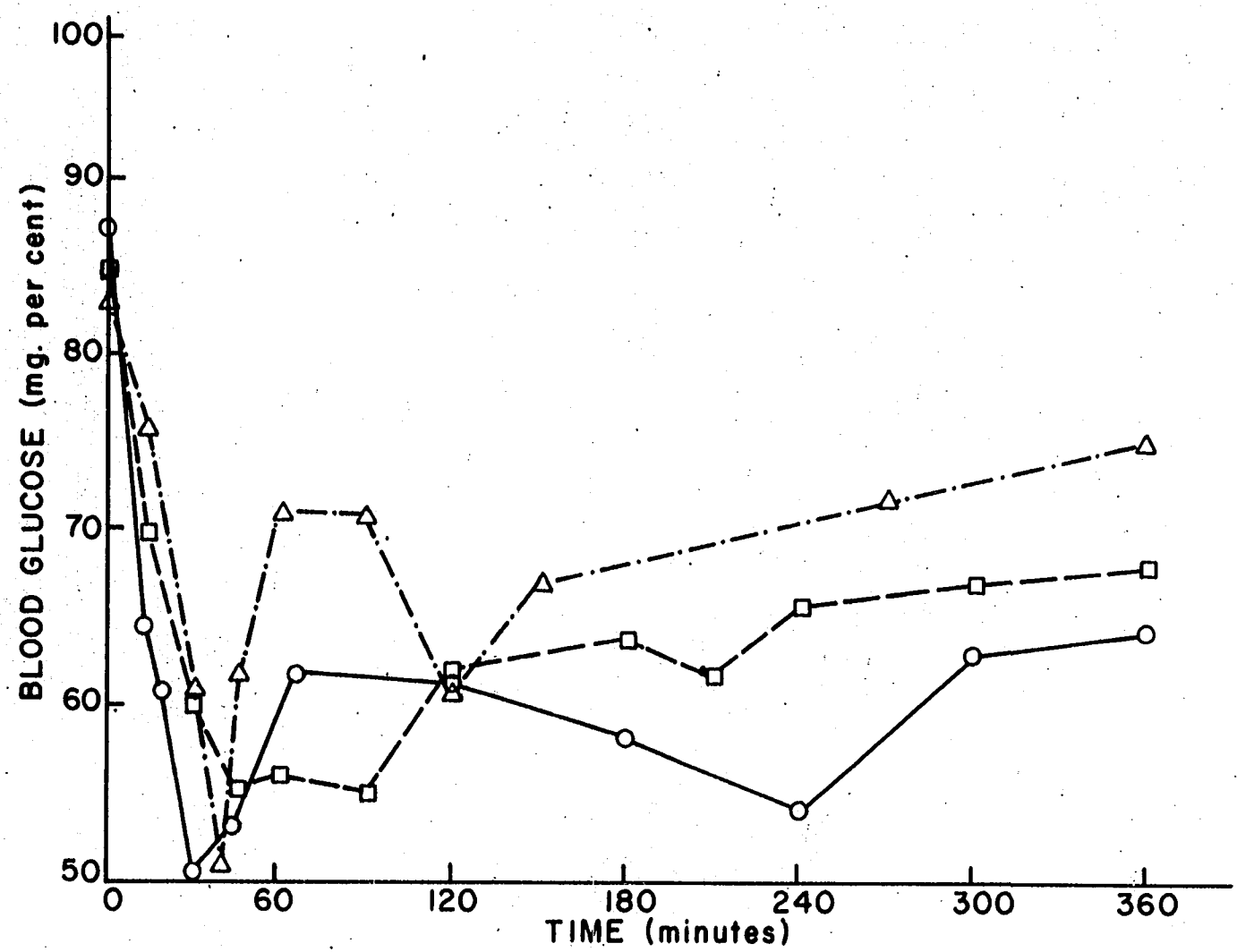

100
90
80
70
60
50
BLOOD GLUCOSE (mg. per cent)
0
60
120
180
240
300
360
TIME (minutes)

Figure 22. Changes in blood glucose concentrations in dogs (H, I, J) after injection of 0.10 gram/kilogram of glucose

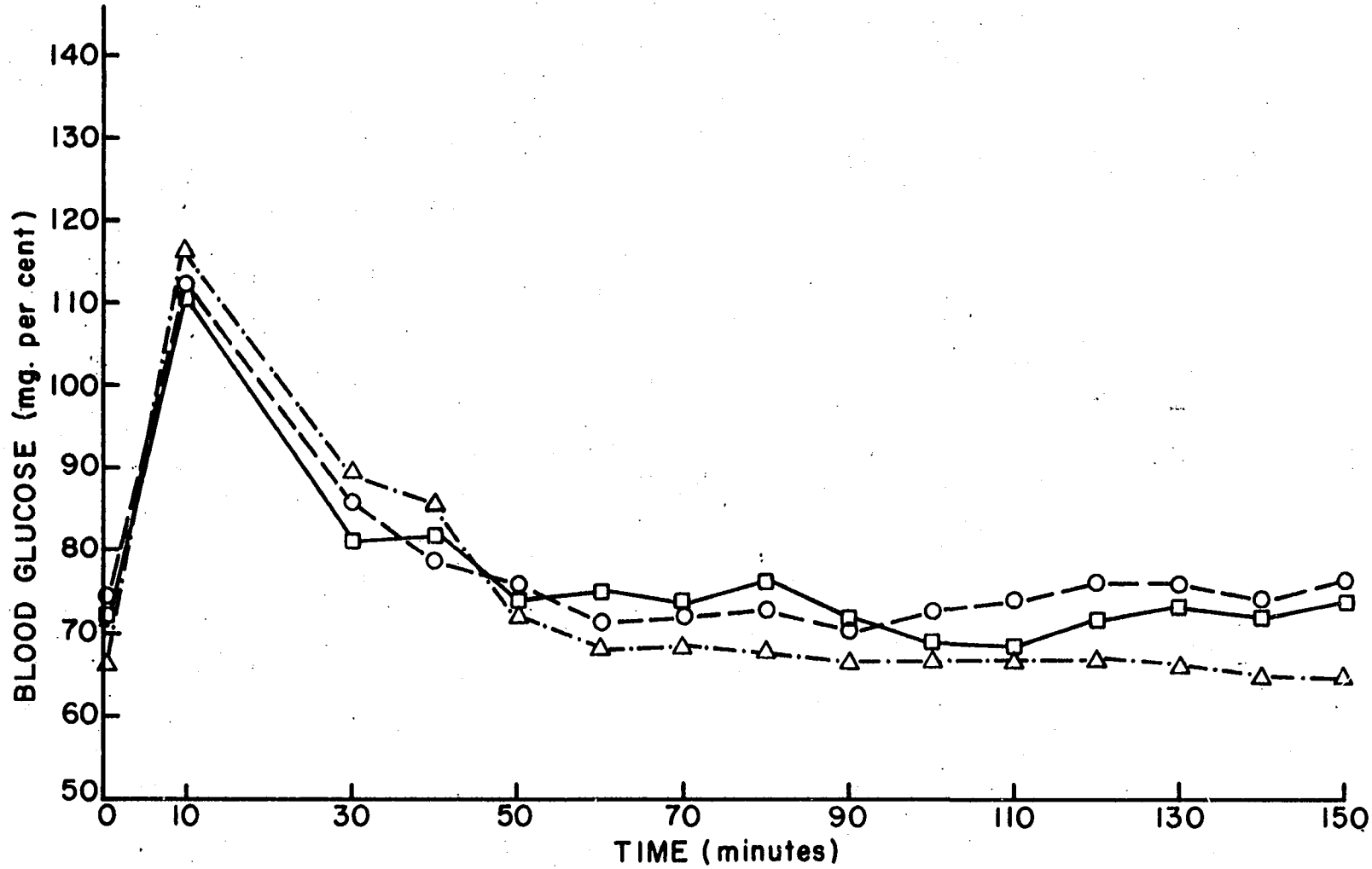

BLOOD GLUCOSE (mg. per cent)
140
130
120
110
100
90
80
70
60
50
0
10
30
50
70
90
110
130
150
TIME (minutes)

Figure 23. Changes in blood glucose concentrations in dogs (K, L, M) after injection of 0.25 gram/kilogram of glucose

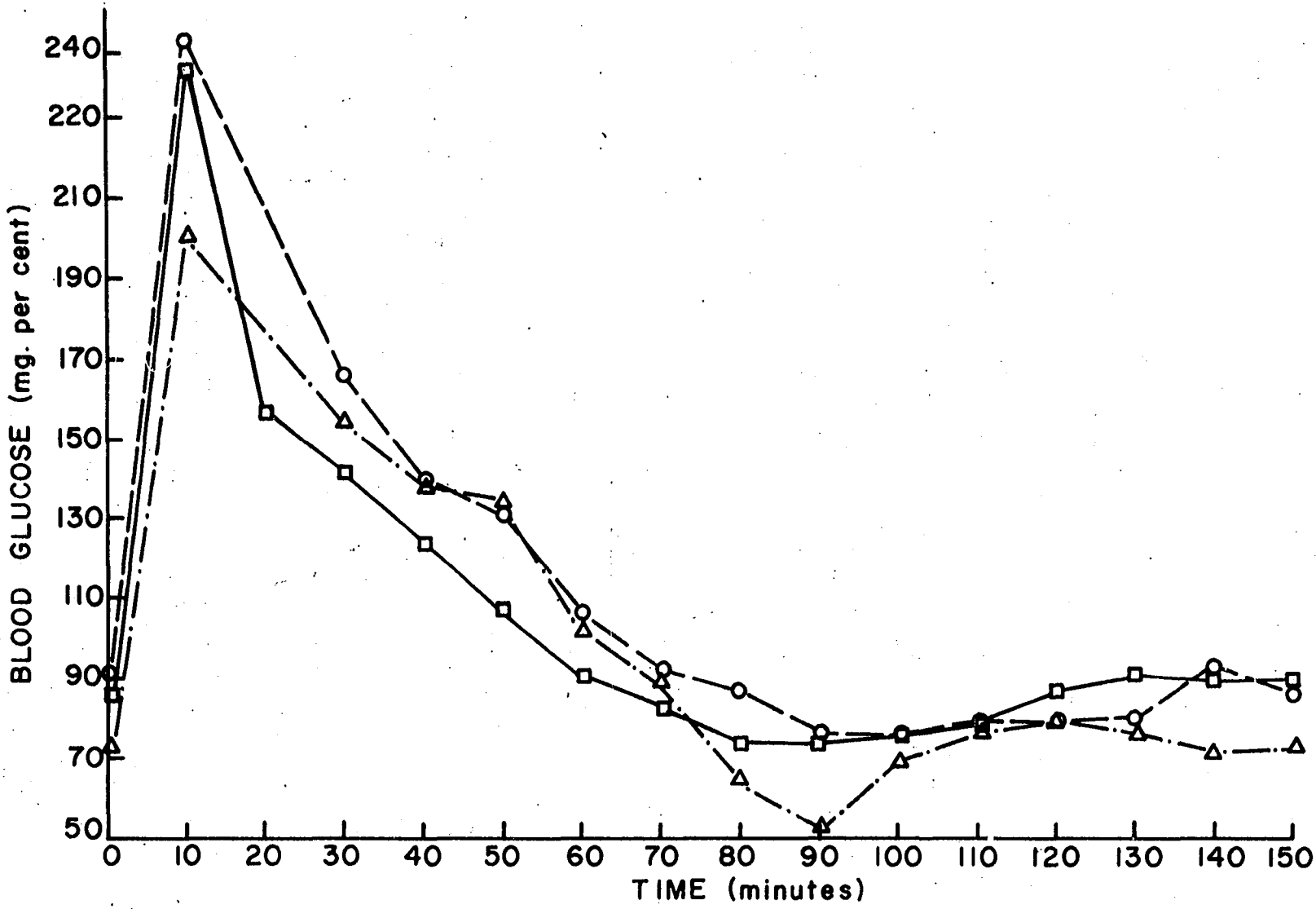

BLOOD GLUCOSE (mg. per cent)
240
220
210
190
170
150
130
110
90
70
50
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
TIME (minutes)

Figure 24. Changes in blood glucose concentrations in dogs (N, O, P) after injection of 0.1 unit/kilogram of insulin with epinephrine infusion sufficient to maintain a 10 mm. Hg rise in blood pressure started 45 minutes after the injection of insulin

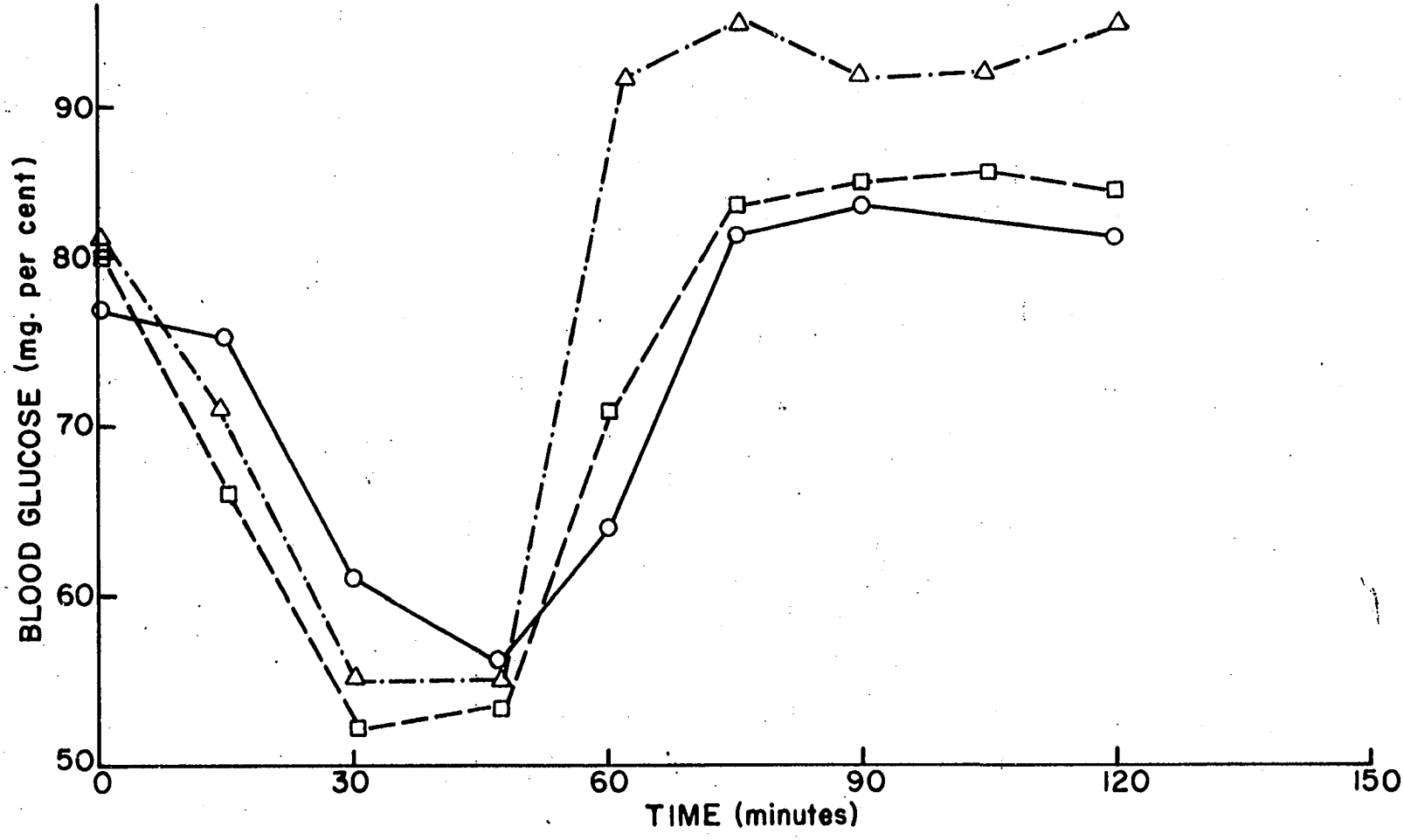
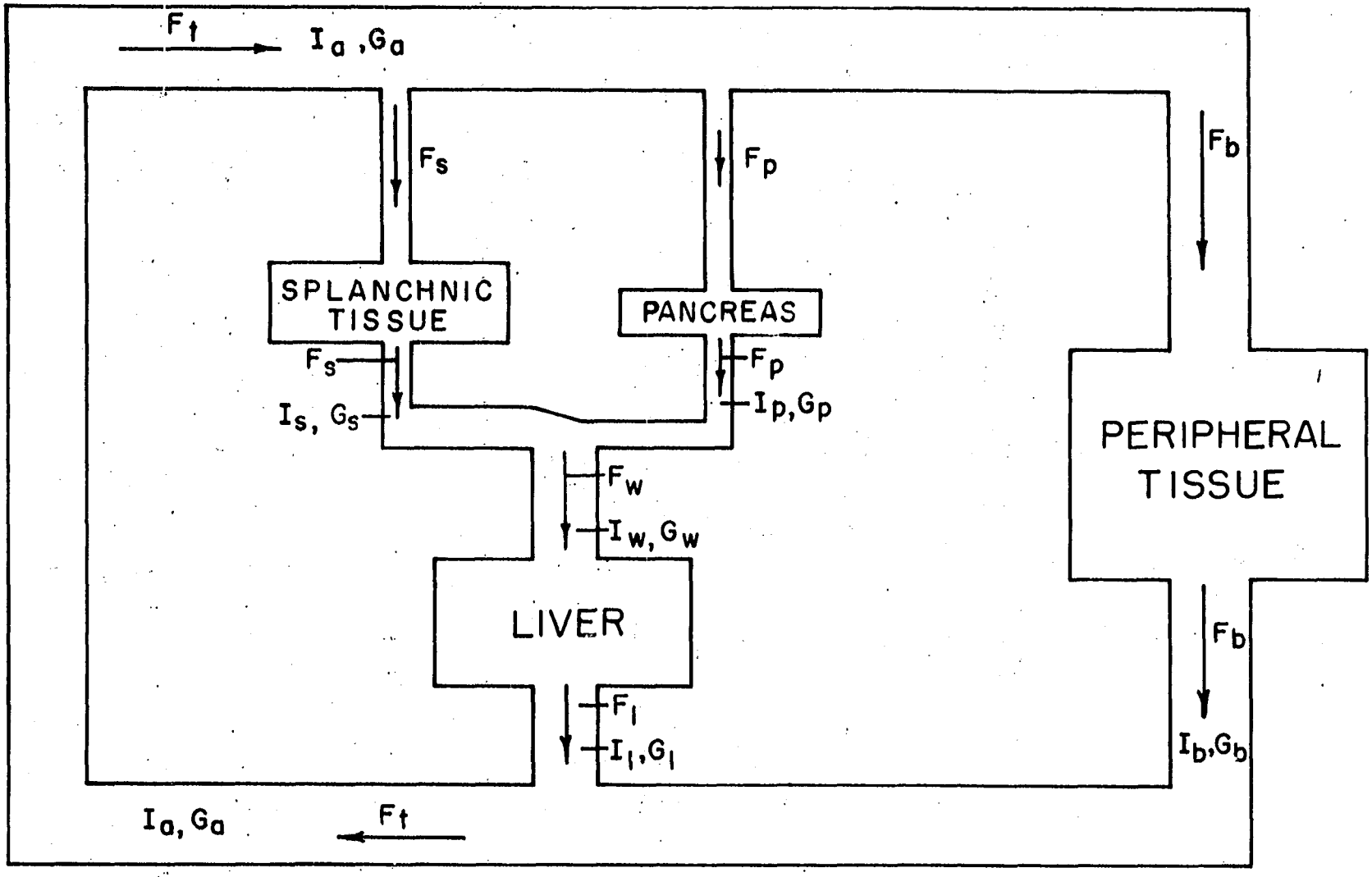

BLOOD GLUCOSE (mg. per cent)
90
80
70
60
50
0
30
60
90
120
150
TIME (minutes)

Figure 25. Schematic diagram of circulation system for multi-compartment model (symbols defined in table 6)


$F_t$
$I_a, G_a$
$F_s$
$F_p$
$F_b$
SPLANCHNIC TISSUE
PANCREAS
$F_s$
$I_s, G_s$
$F_p$
$I_p, G_p$
PERIPHERAL TISSUE
$F_w$
$I_w, G_w$
LIVER
$F_b$
$F_l$
$I_l, G_l$
$I_b, G_b$
$I_a, G_a$
$F_t$