

AN ABSTRACT OF THE THESIS OF

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Title: RATE OF CATABOLIC PATHWAYS OF GLUCOSE IN THE
INTACT RAT

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Virtually nothing is known regarding the quantitative significance of the several catabolic sequences involved in the breakdown of glucose in the intact rat. Studies in this and other laboratories have shown that the Embden-Meyerhof-Parnas pathway, the pentose phosphate pathway and the glucuronic acid pathway are operative in several tissues of the rat. Work in other laboratories has revealed that the pentose cycle and presumably the glucuronic acid cycle operate to a minor extent in several tissues. In the present study, the primary pathways of glucose catabolism in the intact rat were identified and their relative participation evaluated.

In order to evaluate the relative participation of the concurrent operation of the several catabolic pathways of glucose in the intact rat a novel method has been employed. This method, which is referred to as the catabolic rate method, is based upon high resolution

radiorespirometry. The respiratory $^{14}\text{CO}_2$ was determined by means of an ion-chamber-electrometer assembly. Rats were infused continuously for a ten hour period with specifically labeled ^{14}C -glucose substrates. Under these conditions a metabolic steady state with regard to glucose utilization was realized. Thus, data on the interval $^{14}\text{CO}_2$ yield allows one to calculate the catabolic rates of the individual glucose pathways directly. These rates have never been directly measured before. A cross comparison of the rates of individual pathways provide one with information on the relative participation of these glucose pathways, information which has heretofore been unavailable for any biological system.

By the use of the catabolic rate method, it was concluded that in the intact rat, glucose is catabolized predominantly by way of the Embden-Meyerhof-Parnas pathway, to the extent of approximately 83%. The pentose phosphate and the glucuronic acid pathways play a lesser but significant role in the overall catabolism of glucose, accounting approximately for 11% and 6% respectively.

Not only has valuable information been obtained on the fundamental role played by these catabolic sequences but in addition a method has been developed which will allow one to probe more deeply into regulatory mechanisms operative in the intact rat in such areas as biochemical toxicology, endocrinology and etc. This method should also find extensive application in the study of carbohydrate metabolism in health and disease.

Rate of Catabolic Pathways of Glucose
in the Intact Rat

by

John Robert Prince

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TABLE OF CONTENTS

	<u>Page</u>
I. Introduction	1
II. Materials	17
Experimental Animals	17
Radiochemicals	17
Other Chemicals	17
Instrumentation and Other Equipment	18
III. Methods	21
Implantation of Cannulae	21
Substrate Administration	21
Determination of Specific Activity of Blood Glucose	23
Determination of Radioactivity in Lipid Fractions	24
Determination of Radioactivity in Liver Glycogen	25
IV. Results and Discussion	26
Optimal Substrate Level	27
Continuous Infusion Experiments	34
Determination of Pathway Participation	38
Bibliography	56

LIST OF TABLES

<u>Table</u>		<u>Page</u>
I	Incorporation of Infused Glucose-3- ¹⁴ C or Glucose-3, 4- ¹⁴ C into Rat Constituents	33
II	Integrated Yields of Glucose Radioactivity in Respiratory CO ₂	36
III	Estimation of Relative Participation of Catabolic Pathways of Glucose Using Radiorespirometric Data Obtained in Infusion Experiments	51

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Catabolic Pathways of Glucose in Mammals	8
2	A Block Diagram of the Radiorespirometric System	20
3	Metabolism Chamber Designed to Accommodate Experiments Involving Substrate Administration by Means of Continuous Intravenous Infusion	22
4	Rates of $^{14}\text{CO}_2$ Production from Rats Utilizing Glucose-3(4)- ^{14}C Administered by Continuous Intravenous Infusion	32
5	Rates of $^{14}\text{CO}_2$ Production from Rats Utilizing ^{14}C -Specifically Labeled Glucose Substrates Administered by Continuous Intravenous Infusion	35
6	Rates of $^{14}\text{CO}_2$ Production from Rats Utilizing ^{14}C -Specifically Labeled DL-Glutamic Acid Administered by Continuous Intravenous Infusion. Unlabeled Glucose was Infused as Co-substrate at a Rate of 150 mg/hr.	48

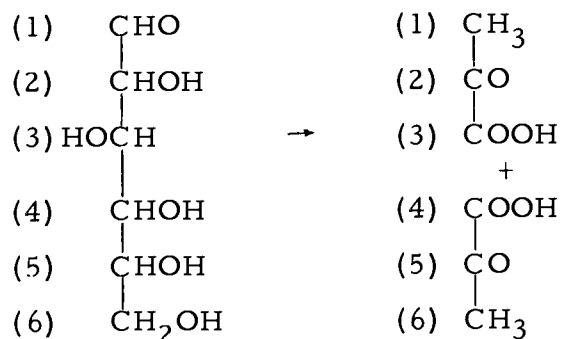
RATE OF CATABOLIC PATHWAYS OF GLUCOSE IN THE INTACT RAT

I. INTRODUCTION

Within the past 30 years much progress has been made in understanding the metabolism of glucose in various biological systems. Of particular interest is the recognition of several major pathways of glucose catabolism, each involving the sequential operation of a number of reactions leading from glucose to various intermediates and eventually to respiratory CO_2 . These pathways are the mechanisms in producing energy by means of biochemical oxidation and in providing intermediates for biosynthesis.

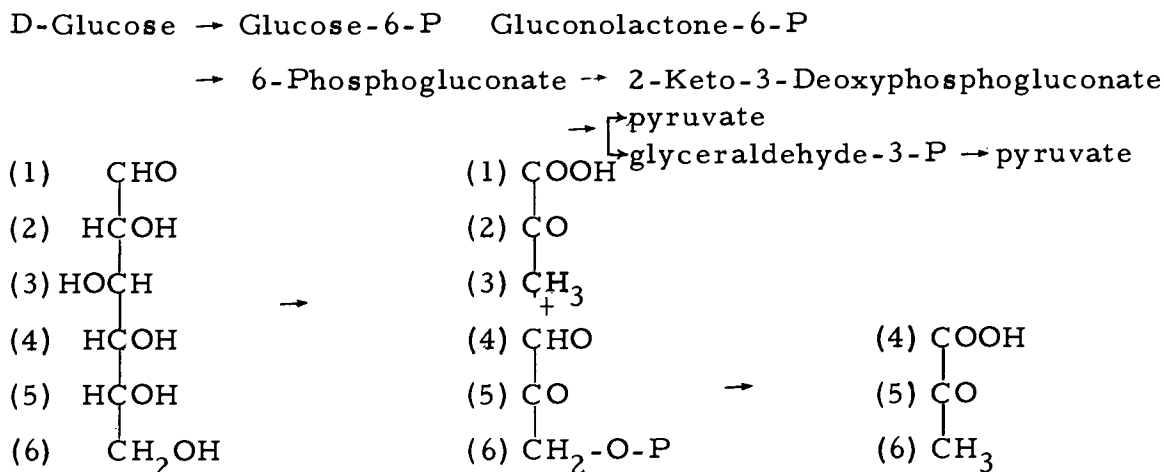
The recognition of the Embden-Meyerhof-Parnas (EMP) pathway represents a major breakthrough in modern biochemistry through the efforts of such eminent scientists as Buchner, Harden, Young, Robison, Meyerhof, Neuberg, Embden, Parnas, Needham, the Coris and Warburg (2). The EMP pathway is, in essence, a symmetrical glycolytic mechanism giving rise to the formation of two moles of pyruvic acid from one mole of glucose. Key steps involved under this pathway are given in the following:

D-Glucose \rightarrow Glucose-6-Phosphate \rightarrow Fructose-6-Phosphate
 \rightarrow Fructose-1, 6-Diphosphate \rightarrow Triose Phosphates
 \rightarrow 1, 3-Diphosphoglycerate \rightarrow 3-Phosphoglycerate
 \rightarrow 2-Phosphoglycerate \rightarrow Phospho-Enol Pyruvate \rightarrow Pyruvate



The pyruvate so formed can be further decarboxylated oxidatively giving rise to the formation of acetyl CoA, the entry compound to the tricarboxylic acid (TCA) cycle pathway. The EMP pathway is by far the most important mechanism for glucose catabolism in biological systems. It is known to occur in animals, plants and a great variety of microorganisms.

In 1952, studies on the glucose catabolism in Pseudomonas species resulted in the elucidation of the Entner-Doudoroff (ED) pathway (10,23). The ED pathway is, in essence, an unsymmetrical glycolytic sequence and is known to occur only in certain species of microorganisms. The key steps of the ED pathway are given in the following:



Thus, one mole of glucose is degraded via the ED pathway giving rise to two moles of pyruvate which can be in turn decarboxylated giving rise to acetyl CoA.

The discovery of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (22, 34, 35) in the middle 1930's paved the ground for the recognition of the pentose phosphate (PP) pathway and the pentose cycle (PC) pathway in 1953 through the efforts of Horecker, Racker and their associates (12, 26). Via the PP pathway, glucose is converted to CO₂ and pentose phosphate. The latter is in turn converted by way of a number of intermediates to fructose-6-P. Fructose-6-P so formed can be catabolized via the EMP pathway through the intermediary formation of fructose-1,6-diP, giving rise to the formation of pyruvate. Such a sequence can therefore be visualized as the PP-EMP pathway and the essential steps are summarized in the following:

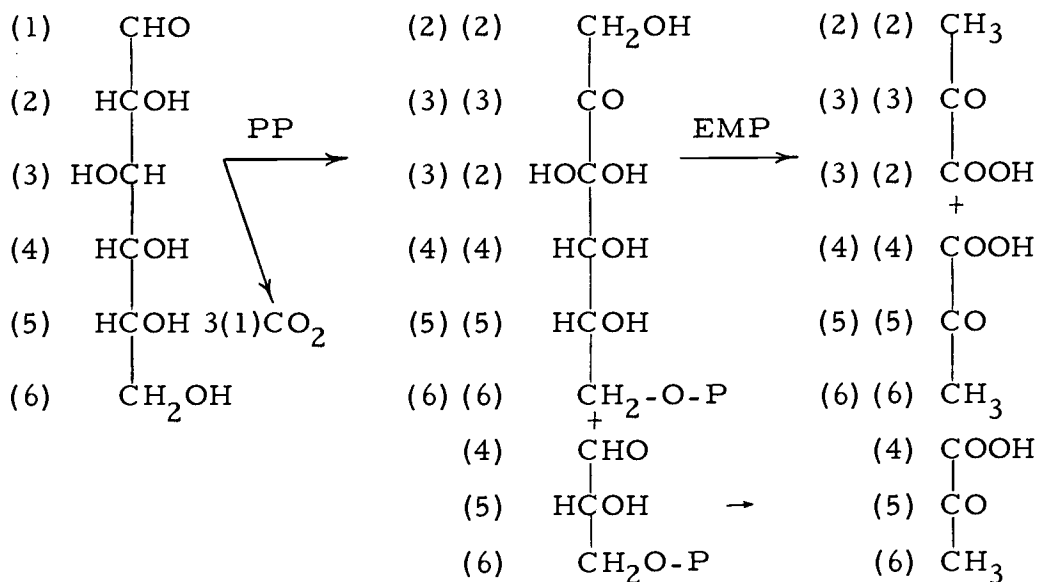
D-Glucose \rightarrow Glucose-6-P \rightarrow Gluconolactone-6-P

\rightarrow 6-Phosphogluconate

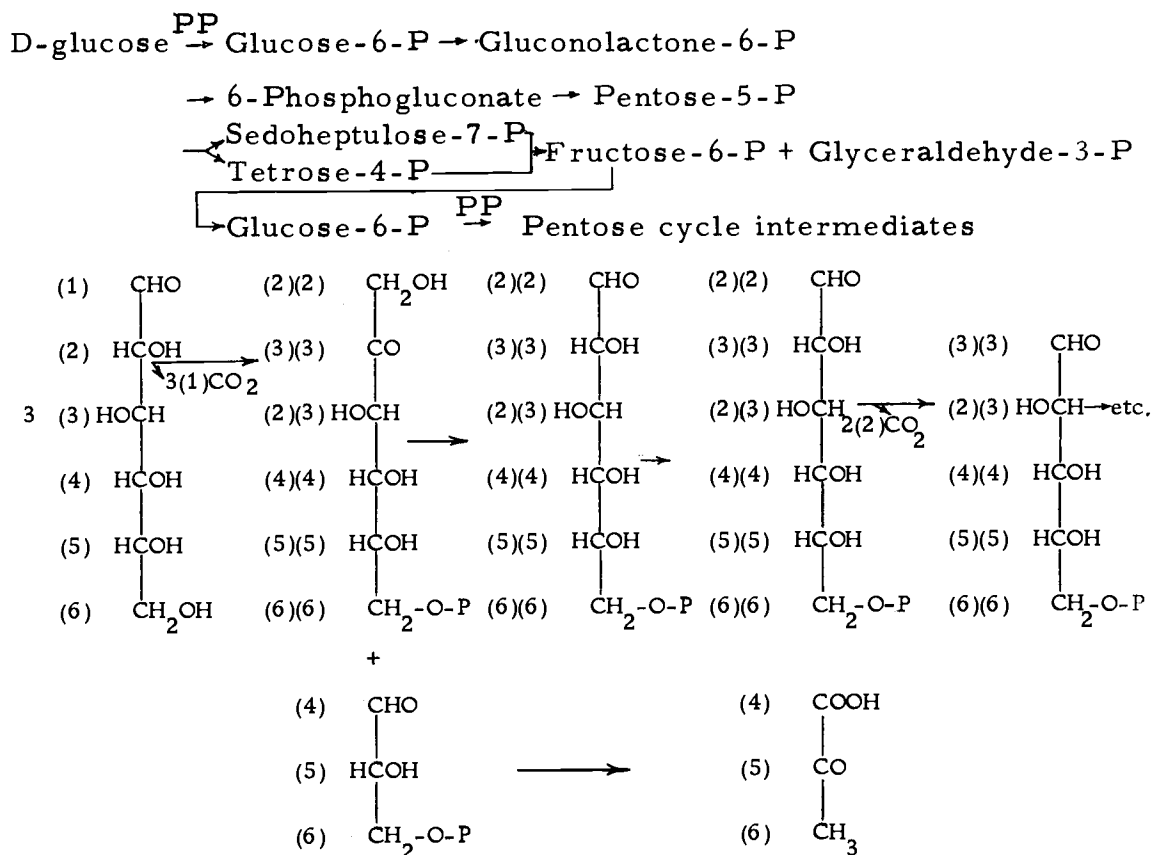
\rightarrow Pentose-5-P \leftarrow Sedoheptulose-7-P
 \leftarrow Tetrose-4-P

\rightarrow Fructose-6-P $\xrightarrow{\text{EMP}}$ Fructose-1, 6-diP \rightarrow Pyruvate

\rightarrow Glyceraldehyde-3-P \rightarrow Pyruvate



Alternately, fructose-6-P, derived from glucose via the PP pathway, can be converted to glucose-6-P via the action of D-glucose-6-phosphate ketol-isomerase. The extent of conversion is naturally dependent on reaction equilibria among various competing mechanisms. The glucose-6-phosphate so formed can be in turn routed once again into the PP pathway; such a sequence of reactions is recognized as the pentose cycle pathway. The key steps involved in the PC pathway and the rearrangement of the carbon skeleton of glucose via this pathway are summarized as follows:



The PP and the PC pathways are known to occur in animals, plants and a great variety of microorganisms. Generally speaking, these two pathways are important for biosynthetic function but are not considered as major mechanisms for energy production except in a few species of microorganisms, notably Acetobacter suboxydans (18). The PC pathway is recognized as an important mechanism in the production of NADPH, a key component in the biosynthesis of fatty acids, etc. (15).

In 1959, the details of the glucuronic acid (GA) pathway were established by Eisenberg and co-workers (9). In essence, via the GA pathway, C-6 of glucose is decarboxylated oxidatively giving rise

The GA pathway is reported to be operative in higher animals (20, 33, 38) and in other biological systems. It is recognized that the GA pathway is of importance for synthesis of L-ascorbic acid in animals other than primates and the guinea pig but is not a major mechanism for energy production.

In mammals, information in the literature (13, 20, 27, 33) indicates that glucose is catabolized via the concurrent operation of three primary pathways, namely: the EMP pathway, the PP pathway (with fructose-6-phosphate formed therefrom routed into the EMP and PC pathways) and the GA pathway, with the latter two presumably playing minor roles insofar as energy production is concerned. Pyruvic acid is the key intermediate derived from glucose via either of these pathways. Oxidative decarboxylation of pyruvate leads to the formation of acetyl CoA which can be readily catabolized via the TCA cycle pathway giving rise to carbon skeletons of various lengths for biosynthetic functions and to respiratory CO_2 and water for energy production. The composite mechanism for glucose catabolism in higher animals is depicted in Figure 1.

Recognition of the nature of catabolic pathways of glucose in biological systems prompted many workers to estimate relative roles played by the individual catabolic sequences in over-all catabolism. Such information is of great importance in understanding the functions of each of the glucose pathways. Numerous methods have been devised

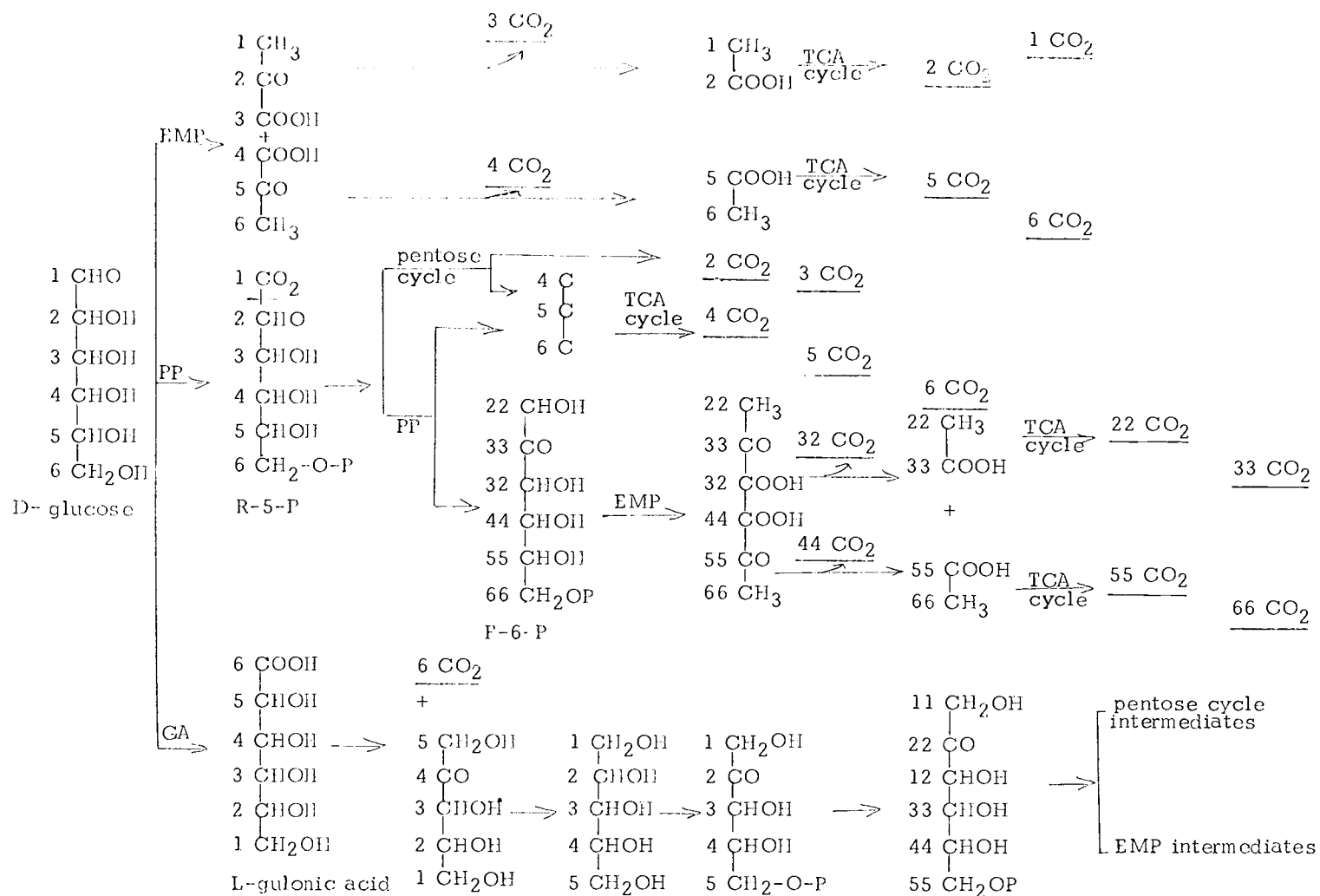


Figure 1. Catabolic pathways of glucose in mammals.

to estimate the participation of glucose pathways making use of radio-tracer methods involving ^{14}C -and ^3H -specifically labeled glucose as substrates. Unfortunately, some early works in this regard did not provide precise information since one of the key assumptions underlying the early methods stated that the pentose phosphate derived from glucose via the PP pathway does not participate further in catabolic functions. Such an assumption was soon proven incorrect upon unveiling of the details of the PC pathway by Horecker (12) and Racker (26). The catabolic fate of pentose phosphate derived from glucose has been taken into consideration by various investigators since 1956 (16, 27, 32, 37, 39) in devising methods for meaningful estimation of glucose pathways.

Existing methods for pathway estimation can be classified into different categories on the basis of either experimental approaches or objectives. Insofar as experimental approaches are concerned, in most of the reported experiments labeled substrates were generally introduced into tissue preparations or intact biological systems in single doses. Information on glucose pathways was obtained by examining one or more of the following manifestations:

- (a) relative specific activities of glucose derivatives such as glycogen, glycerol, lactate, CO_2 , etc. derived from ^{14}C -specifically labeled glucose substrates (16, 27),
- (b) yields of ^{14}C in fatty acids, lactate, etc. derived from

^{14}C -specifically labeled glucose substrates (16), and
 (c) yields of ^{14}C respiratory CO_2 derived from ^{14}C -specifically
 labeled glucose substrates (17, 32).

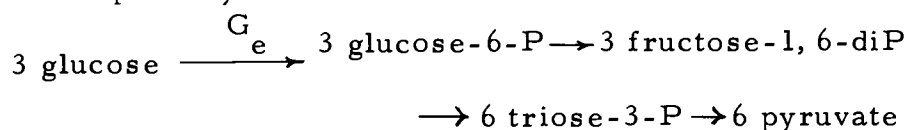
Practically all the reported works on glucose pathways are concerned with the relative participation of the EMP pathway and the PP (or PC) pathway in biological systems. This is understandable since these two pathways are by far the most common mechanisms in animals, plants and a majority of the microorganisms. The participation of other pathways such as the GA pathway in animals and plants has been investigated by some workers. However, up to the present time, there exists no useful method for the estimation of three concurrent glucose pathways in any biological system.

The objectives of the existing methods for the estimation of two concurrent pathways have been focused on a direct assessment of the contribution of the PP (or the PC) pathway. The contribution of the EMP pathway is deduced from the difference between total amount of glucose metabolized and the fraction of glucose engaged in PP (or PC) pathway. This approach is naturally under the assumption that substrate glucose does not participate in either anabolic pathways or other catabolic pathways. Wang and his co-workers (30, 32) emphasized the importance of understanding the initial fate of substrate glucose when subjected to catabolic processes. The objective of their method is to estimate the contribution of the PP pathway in over-all

glucose metabolism. The catabolic fate of substrate glucose in a biological system equipped with the EMP and the PP pathway is envisioned by these workers as follows:

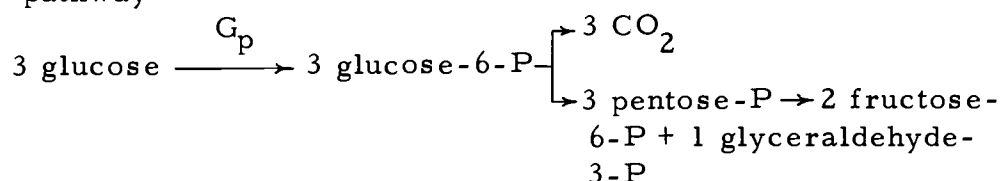
via the EMP pathway: G_e = fraction of glucose catabolized via

the EMP pathway



via the PP pathway: G_p = fraction of glucose catabolized via the

PP pathway

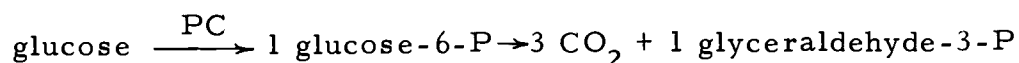
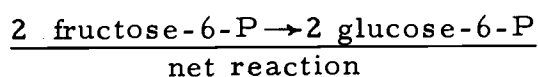
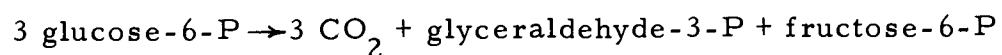


It is recognized that fructose-6-phosphate, derived from glucose via the PP pathway can be catabolized either by the PC pathway or the EMP pathway.

On the other hand, Katz and Wood (16, 39) have focused the objective of their method to the assessment of the net participation of the PC pathway. They defined the PC pathway as:

via the PC pathway: PC = fraction of glucose catabolized via

the PC pathway



More recently, Landau, Katz and Bartsch have examined in more detail the validity of their method in applying to studies with adipose tissue, taking into consideration the equilibria of several key steps in glucose catabolism such as triose phosphate isomerization (15) and the transaldolase reaction (19). It should be noted that the contribution of the pentose cycle pathway designated as PC and the term G_p , defined by Wang and his co-workers for the participation of the PP pathway in over-all glucose catabolism, can be readily related to each other (17, 32) and represented by the expression: $G_p = \frac{3 \text{ PC}}{1 + \text{PC}}$

Whereas the existing methods for pathway estimation do provide one with useful information on the relative roles of two major pathways operating concurrently in biological systems, these methods are nevertheless suffering from several drawbacks. Thus, in order to assess the role played by the PC pathway, Katz and his co-workers assumed that glucose is not engaged to any great extent in non-triose phosphate pathways and that the pentose phosphate, derived from the glucose via the PP pathway, is not engaged in any biosynthetic processes (39). The validity of these assumptions cannot be readily verified. Of more importance is the fact that the existing methods do not provide one with a direct assessment on the participation of the EMP pathway. Only by assuming that glucose is not engaged in any anabolic processes and that the EMP and the PP pathways are the only catabolic routes can one estimate the contribution of the EMP pathway

by difference. The validity of these assumptions may be questioned since it is known that glucose can be incorporated into glycogen, etc. and that the glucuronic acid pathway is operative in several biological systems including higher animals. It is for this reason that in reality there exists, at the present time, no defined information on the relative participation of glucose pathways in mammals.

The experimental approaches underlying various existing methods for pathway studies with mammals are also matters of concern. Firstly, reported works on glucose pathways in mammals using radiotracer methods are those involving the use of either tissue preparation or intact animals. Findings with a preparation of a given tissue do not reflect the catabolic behavior of intact animals. Secondly, means for substrate administration in reported experiments with intact animals can be classified into two basic types on the basis of the routes used for substrate administration. A number of workers prefer to administer labeled glucose by means of single-dose intravenous injection of a trace amount of labeled glucose. Findings in this type of experiment reflect at best only the catabolic behavior of endogenous glucose. It is likely that a trace amount of labeled glucose, once injected into the circulatory system, is trapped by various endogenous pools such as glycogen, and thereby creating a distorted catabolic picture. Wang and co-workers (33) administered 1.5 gm of ^{14}C -labeled glucose, in solution form, to fasted rats by

means of stomach tubing. Although they have obtained some useful radiorespirometric data, their findings do not permit them to assess quantitatively the relative participation of catabolic pathways. In fact, single-dose substrate administration, regardless of the route, constitute a transient increase of glucose in the animal body. Findings obtained in this type of experiment would not provide one with meaningful kinetic information leading to a correct understanding of the catabolic fate of glucose.

If these objectives are satisfactorily met, valuable information will be obtained on the fundamental role played by these catabolic sequences. In addition, future experiments can be designed which will allow one to probe more deeply into regulatory mechanisms operative in the intact rat in such areas as biochemical toxicology, endocrinology, etc.

The choice of the intact animal as a suitable biological test system may be justified in several ways. While not minimizing experimental work with in vitro systems, it is true that before a coherent picture will emerge from such studies, experiments will have to be performed on intact biological systems.

Apart from the desirability of using an intact animal as a biological test system, there are conditions where the experimenter has no choice. An important example may be found in the field of nuclear medicine (1, 24, 29). Recognizing the importance of basic

understanding on carbohydrate metabolism in health and disease (7) it is reasonable to assume an even greater role for radiorespirometric studies of metabolism in humans will be attained in the future.

It should be noted however that because of the complexity of a system like an intact rat, it will be necessary to make certain simplifying assumptions in order to obtain a reasonable solution to the problem. While the assumptions are considered to be physiologically and biochemically sound, their complete validity may need further experimental verification.

With this general information as background this study was undertaken with the following specific objectives in mind:

1. To detect the operation of the individual pathways of glucose catabolism in a complex biological system, specifically the rat.
2. To improve the existing methodology for the study of glucose catabolism in the intact rat.
3. To estimate the relative participation of the three primary reaction sequences for glucose degradation in this biological test system.

In the present work, glucose catabolism in intact rats is studied by means of the radiorespirometric method. Carbon-14 specifically labeled glucose at an optimal level, as determined in a series of preliminary experiments, was administered to rats

continuously by way of intravenous infusion. Data obtained on the rate of $^{14}\text{CO}_2$ production from rats utilizing labeled glucose under steady state conditions was used to calculate the catabolic rates of three concurrent pathways; i. e., the EMP pathway, the PP pathway and the GA pathway.

II. MATERIALS

Experimental Animals

Male Sprague Dawley rats obtained from the Berkeley Pacific Laboratories, Berkeley, California, were used in all phases of this work. The animals were obtained at an average weight of 220 gm, and were maintained on Purina Laboratory Chow and water, ad libitum.

Radiochemicals

The radiochemicals used in the present work were obtained from the New England Nuclear Corporation, Boston Mass. Identity and purity of each of the radiochemcials was established by paper chromatography and autoradiography. Labeling positions for each of the radiochemicals were established by means of microbial degradation of the respective compounds.

Other Chemicals

Reagent grade chemicals were used in all operations. Materials for liquid scintillation counting were obtained from the Packard Instrument Company, Downer's Grove, Illinois. Glucostat reagent for the determination of blood glucose was obtained from the Worthington Biochemical Company, Freehold, New Jersey.

Instrumentation and Other Equipment

Polyethylene-silicone rubber cannulas were prepared for these studies by Mr. J. J. Krake of the Upjohn Research Laboratory, Kalamazoo, Michigan, according to the methods described by Heatley and Weeks (36).

The infusion system consists of a Harvard Model 600-2-200 syringe pump with variable control (Harvard Apparatus Co., Dover, Massachusetts) modified to drive four 10 ml plastic disposable syringes (Plasti-pak, Becton Dickinson Co., E. Rutherford, New Jersey). A 30-inch length of PE 100 tubing (Clay-Adams, Inc., New York) is attached to each syringe for connection with the indwelling cannula of the rat.

Respiratory $^{14}\text{CO}_2$ was measured continuously by means of a radiorespirometric system described in detail by Wang (31). The 1.6-liter animal chamber is swept with air at a rate of 500 ml per minute, carrying respiratory $^{14}\text{CO}_2$ through a drying column and then a one liter flow-ion chamber. The ion current generated by beta particles arising from the disintegration of ^{14}C in the ion chamber was measured by a vibrating reed electrometer (Cary Model 31, Applied Physics Co). The digital pulses were accumulated on a six decade RIDL Model 49-43 scaler, and printed out at pre-set time intervals upon receipt of command from a programmer (RIDL 52-44) and timer (RIDL 54-8), which also control simultaneously the operation

of three other identical systems. The calibration of ^{14}C -
measurement in the ion chamber has been described previously (8).
A block diagram of the radiorespirometric system is given in Figure
2.

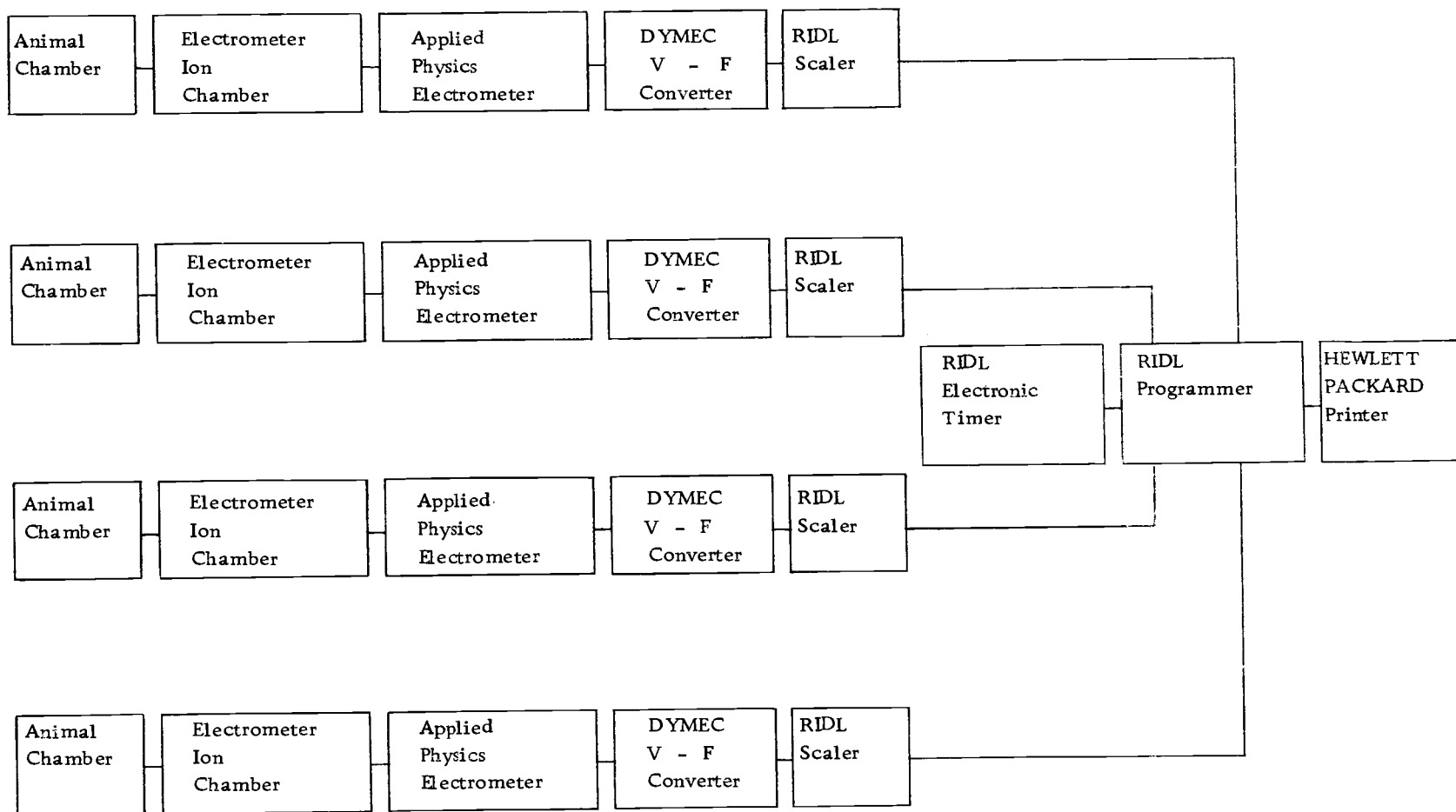


Figure 2. Block Diagram of Animal Radiorespirometer with Printout System.

III. METHODS

Implantation of Cannulae

The indwelling cannula was placed in the anterior vena cava, usually through the right jugular vein, according to the technique of Weeks and Davis (36). The animals were allowed to recover at least five days after surgery, or until the presurgical weight of 250 to 280 gm was regained. When used experimentally, the animals usually weighed 280 to 320 gm.

Substrate Administration

Rats were prepared for continuous infusion in the following steps: Food was removed 2 to 3 hours after the beginning of the daily 12-hour dark period and 12 hours later the animal was given 1,500 mg glucose in 3 ml water by stomach tubing. Six hours after this feeding, the rat was considered to be in a post-absorptive state (33) with maximal liver glycogen storage. At this time the animal was placed in a restrainer cage (Aerospace Industries, Model 1R350, Garnerville, New York) and connected to the substrate syringe which had been filled with the substrate solution and pumped sufficiently to fill the connecting line and purge all bubbles. The animal, in its restraining device, was then inserted into a 4" x 9" radiorespirometer chamber (Figure 3).

Metabolism chamber and restraint cage
for continuous infusion of labeled substrates
(chamber support, face plate clamp and
water supply not shown)

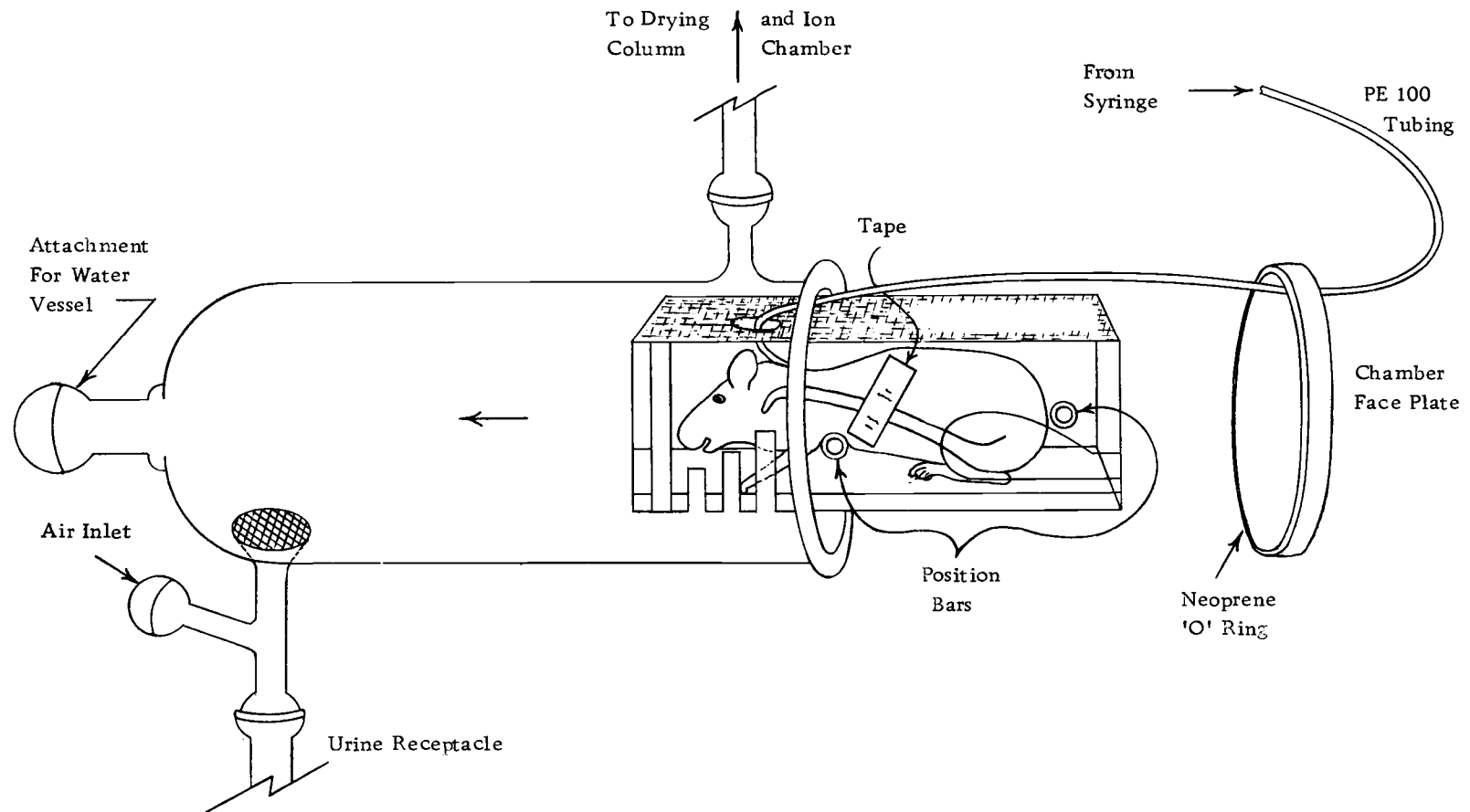


Figure 3. Metabolism chamber designed to accommodate experiments involving substrate administration by means of continuous intravenous infusion.

In all but exploratory experiments, the infusion was begun and maintained at a rate of 0.84 ml per hour with glucose concentration adjusted to the appropriate level so that 150 mg glucose, containing 0.01 to 0.02 cc ^{14}C , was administered to the rat per hour. Measurement of respiratory $^{14}\text{CO}_2$ was continued through at least 5 hours after the termination of infusion, at which time the radioactivity in respiratory CO_2 had subsided to a negligible level.

Determination of Specific Activity of Blood Glucose

In several experiments it was desirable to know the specific activity of blood glucose while the rat was metabolizing infused glucose at the metabolic steady state. The substrate infusion was stopped at the end of six hours and blood was collected by heart puncture 30 to 45 seconds after the implanted cannula was disconnected. A 0.2 ml aliquot of the blood sample was analyzed for glucose using the glucostat method.

To determine the radioactivity of the blood sample, one ml blood was deproteinated with 2 ml of trichloroacetic acid and the mixture centrifuged for several minutes. One ml of the supernatant was placed in a 20 ml liquid scintillation counting vial and agitated with a stream of nitrogen gas to remove any residual $^{14}\text{CO}_2$. Ten ml of Bray's solution (6) was added to the counting vial and the contents were thoroughly mixed by shaking. The sample was then

counted in a liquid scintillation counter (Packard Instrument Company, Model 314EX2). A solution containing known amounts of glucose-U- ^{14}C was used as an internal standard to determine the counting efficiency for the described mixture. All counting was carried out over a sufficient duration so that the relative standard deviations of the counting data were less than 1%.

Specific activity of glucose was calculated on the basis of net $^{14}\text{CO}_2$ radioactivity in dpm per mg of glucose. Experimental evidence indicates that radioactivity of blood components other than glucose was negligible.

Determination of Radioactivity in Lipid Fractions

It was desirable to learn the extent of incorporation of ^{14}C from the labeled glucose into lipid fractions of various tissues under the conditions of these radiorespirometric experiments. For this purpose, rats were sacrificed at the end of 6 hours of a typical radiorespirometric experiment. Various tissues were isolated and individually homogenized. The lipid fraction was extracted according to the method of Katz, Landau and Bartsch (15). The individual extracts were evaporated in vacuo and processed for counting in the conventional manner by liquid scintillation counting.

Determination of Radioactivity in Liver Glycogen

The incorporation of glucose-3- ^{14}C and glucose-3(4)- ^{14}C into liver glycogen was determined as a further aid to the interpretation of the respirometry data. Rats were sacrificed at the end of 6 hours of a typical radiorespirometric experiment. After a sharp blow on the head, the liver was quickly extirpated and frozen in a dry ice-acetone mixture. Liver glycogen was extracted by the method of BeMiller (4). Aliquots of the extract were processed for counting in the liquid scintillation counter in the conventional manner.

RESULTS AND DISCUSSION

The objective of the present work was to ascertain the catabolic fate of administered glucose in the rat. To this end, radiorespirometric experiments were designed to collect information that can be used to calculate the catabolic rate of individual glucose pathways. Comparative analysis of the rate data so obtained should provide one with a good understanding on the relative contribution of each of the major glucose pathways.

In the present work, emphasis was focused on the initial fate of exogenous glucose engaged in catabolic processes. It is noted that in glucose catabolism via any one of the three recognized major catabolic routes (Figure 1), certain carbon atoms are converted promptly to respiratory CO_2 . It follows that if one can collect meaningful data on the rate of CO_2 formation from the respective carbon atoms, one should be able to utilize such data to calculate approximate rates of respective catabolic pathways.

Intravenous infusion was chosen as the route for substrate administration in radiorespirometric experiments since by continuous infusion of a solution of labeled glucose at optimal concentration, a metabolic steady state can be readily reached in the rat with respect to the turnover of the exogenous glucose (16). Experimentally, the realization of a metabolic steady state can be verified by determining

the rate of respiratory $^{14}\text{CO}_2$ formation from labeled glucose. Thus, if glucose-3- ^{14}C is used as the substrate, one should expect that the rate of conversion of C-3 of glucose to CO_2 will follow a constant rate soon after the beginning of substrate infusion, reflecting the catabolic steady state. This is true since it is known that the EMP-pyruvate decarboxylation pathway is a major catabolic route in rats and via this route, the conversion of C-3 or C-4 of glucose to CO_2 is a prompt process. Data on rates of CO_2 production obtained with rats metabolizing glucose under steady state conditions are the only ones that can be interpreted and analyzed to gain kinetic information of catabolic pathways.

Optimal Substrate Level

There are several important criteria that should be taken into consideration in designing radiorespirometric experiments involving continuous glucose infusion, particularly with respect to the rate of infusion in terms of amount of glucose administered per unit time. First of all, since one is interested in the catabolic fate of glucose, it will be desirable to ensure that the bulk of the administered glucose, as soon as it enters the circulatory system, is rapidly oxidized to CO_2 . For this reason, the rats used in the experiments were those maintained at a post-absorptive state. Under these conditions, a limited amount of substrate glucose is expected to be incorporated

into liver glycogen. If the glucose is administered to the rat at a rate significantly higher than that of the metabolic capability of the rat, undesirable physiological manifestations may result. Notable ones are the secretion of an excessive amount of insulin and loss of labeled glucose through renal excretion. On the other hand, if the rate of glucose infusion is too low, a distorted catabolic picture, with respect to the exogenous glucose, may result from the interference of endogenous glucose. It is known that with radiotracer experiments, the extent and rate of conversion of labeled carbon atoms of substrate to CO_2 is dependent on the size of the endogenous pool (28).

In order to determine the optimal substrate level, a series of radiorespirometric experiments were carried out using glucose-3 (4)- ^{14}C as the test substrate. Solution of the labeled glucose was infused into the rat at a rate of 0.84 ml/hr. The specific rate of infusion with respect to volume of solution is determined on the basis of physiological and mechanical considerations. The concentrations of substrate solution ranged from 6% to 60% w/v. Hence, the rate of infusion with respect to the amount of glucose administered ranged from 50 mg to 500 mg/hr.

The findings in this series of experiments are given in Figure 4 in which the radioactivities of $^{14}\text{CO}_2$ in dpm collected during 5-minute intervals are plotted against time. In these experiments,

glucose infusion was allowed to continue for a period of 10 hours after which time measurement of respiratory $^{14}\text{CO}_2$ was continued for 5 hours, until the production of respiratory $^{14}\text{CO}_2$ subsided to an insignificant level. Data given represents the average value of at least four replicate experiments and deviation among comparable results is found to be no greater than 4%. The data given in Figure 4 provide one with two types of information: namely, rate of $^{14}\text{CO}_2$ production expressed as dpm per unit time interval and integrated yields of $^{14}\text{CO}_2$ at the end of experiment (5 hours after the termination of infusion) expressed as percent of radioactivity in the administered glucose.

It is noted that when the rate of glucose infusion is as low as 50 mg/hr., only 57% of the radioactivity of the glucose is converted to respiratory CO_2 . Evidently, the relatively small amount of exogenous labeled glucose infused into the rat is not able to reduce significantly the supply of endogenous glucose, lipids and other compounds into the circulatory system. Consequently, a sizable portion of the labeled glucose was trapped in various endogenous pools by way of exchange reactions associated with anabolic processes, resulting in a limited conversion of C-3 and C-4 of glucose to respiratory CO_2 .

On the other hand, when the rate of infusion is 500 mg/hr., one finds that the rate of $^{14}\text{CO}_2$ production reached a constant level only

in the last two hours of the infusion period. Moreover, even after termination of glucose infusion at 10 hours, respiratory $^{14}\text{CO}_2$ was produced at a significant rate over a period of the next 5 hours. The cumulative yield of $^{14}\text{CO}_2$ at the end of the experiment is found to be 67%, only slightly higher than that observed in experiments with infusion rate of 50 mg/hr. Evidently, with such a high rate of substrate administration, the rat is not capable of metabolizing promptly the infused glucose resulting in the channeling of a significant portion of the administered glucose into depletable anabolic reserves.

Comparison of findings in these experiments leads one to believe that the optimal substrate level is when the infusion rate, with respect to amount of glucose, is kept in the neighborhood of 150 mg/hr. Thus, one finds that the cumulative yield of $^{14}\text{CO}_2$ is highest at this substrate level, a fact implying that at this substrate level, the administered glucose can be promptly routed into catabolic processes without too much interaction with the endogenous reserve. This contention is verified by the findings of a series of experiments, using glucose-3- ^{14}C and glucose-3(4)- ^{14}C as substrates, which were designed to determine the specific activity of blood glucose and extent of incorporation of labeled glucose into liver glycogen and lipid fraction. These data were obtained with samples taken from the rat 6 hours after the beginning of glucose infusion at which time a metabolic steady state has been definitely reached in the rat with respect to the

infused glucose (Figure 4).

As shown in Table 1, the specific activity of blood glucose is essentially the same as that of the infused glucose. This fact indicates that at an infusion rate of 150 mg/hr., and under metabolic steady state conditions, the supply of endogenous glucose into the circulatory system has been practically shut off. In other words, glucose metabolism in the rat, under these conditions, involves almost exclusively the exogenous glucose. Of equal interest is the finding that the amount of labeled glucose incorporated into liver glycogen is very limited in extent. This fact reveals that with the rat at post-absorptive state, little infused glucose is incorporated into anabolic reserves. Similarly, the incorporation of the labeled carbon atoms and hence the trioses, derived from infused glucose, into lipid fraction is also found to be very limited in magnitude. It is of interest to note that the extents of incorporation of infused glucose to lipid fraction are found to be approximately the same in either the glucose-3-¹⁴C or the glucose-3(4)-¹⁴C experiments. It therefore appears that there exists a rapid equilibration between glyceraldehyde-3-P and dihydroxyacetone phosphate, derived from substrate glucose, by the action of triose isomerase.

It should be noted further that when the infusion rate is 150 mg/hr., the total administered dose of glucose over a period of 12 hrs. is 1,800 mg, an amount of the same magnitude as the daily food

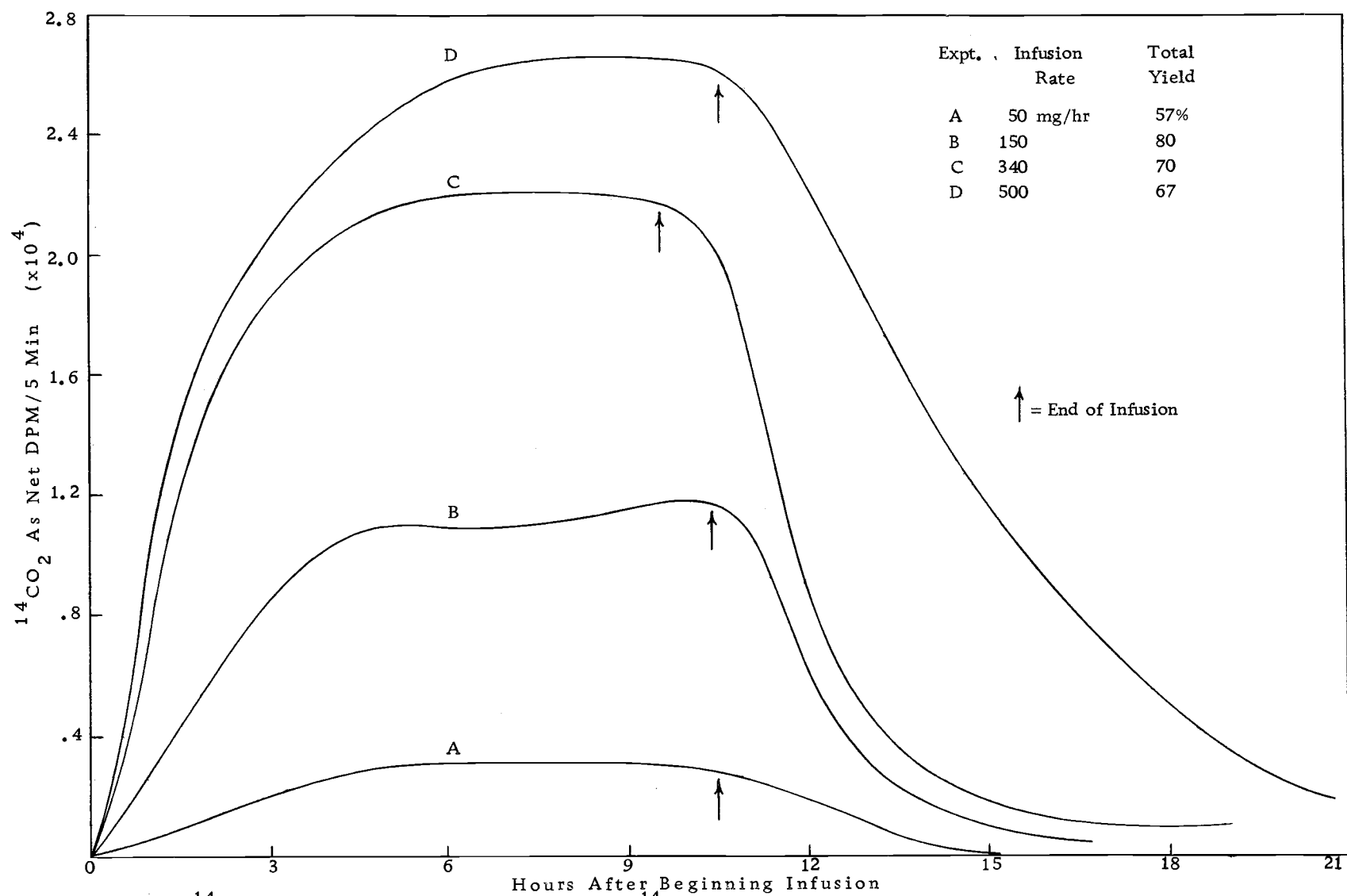


Figure 4. Rates of $^{14}\text{CO}_2$ production from rats utilizing glucose-3(4)- ^{14}C administered by continuous intravenous infusion.

Table I. Incorporation of Infused Glucose-3-¹⁴C or Glucose-3(4)-¹⁴C into Rat Constituents.

	Glucose-3- ¹⁴ C	Glucose-3(4)- ¹⁴ C
Blood glucose, mg/ml		0.67
Sp. act. of infused glucose, dpm/mg		1140
Sp. act. of blood glucose, dpm/mg		1138
Glycogen, % of infused glucose	1.3	1.4
Lipids in: (% of infused glucose)		
Adipose	0.22	0.09
Brain	0.13	0.10
Lung	0.09	0.13
Heart	0.09	0.06
Kidney	0.15	0.15
Muscle	0.08	0.07

Labeled glucose substrates were infused into the rat at a rate of 150 mg/hr. Samples for analyses were taken 6 hrs. after the beginning of the infusion process.

intake of the rat. At this infusion rate, and assuming that the infused glucose is promptly metabolized by the rat, the utilization rate of glucose in rats of the weight range used in the present studies is only slightly less than that reported by Baker, et al. (3).

Continuous Infusion Experiments

A series of radiorespirometric experiments using various specifically labeled glucose infused into the post-absorptive rat at the optimal infusion rate were then carried out. The findings are given in Figure 5 in which the rates for the production of respiratory $^{14}\text{CO}_2$, expressed in dpm per 5-min. intervals, were plotted against time. Given in Table II are integrated yields of CO_2 from the labeled carbon atoms at the end of experiments; i. e., 15 hours after the beginning of infusion and 5 hours after the termination of infusion. Data shown represent the average of at least four replicate experiments with deviations in results among replica experiments being less than 4%.

The radiorespirometric data given in Table II and Figure 5 provide one with much information on the fate of carbon atoms of glucose in rat catabolism. As shown in Figure 5, the rates for the conversion for most of the carbon atoms of glucose to respiratory CO_2 followed an ascending slope during the first few hours of glucose infusion and reached constant levels until the substrate infusion was terminated, except in the case of C-4 of glucose, a mild ascending

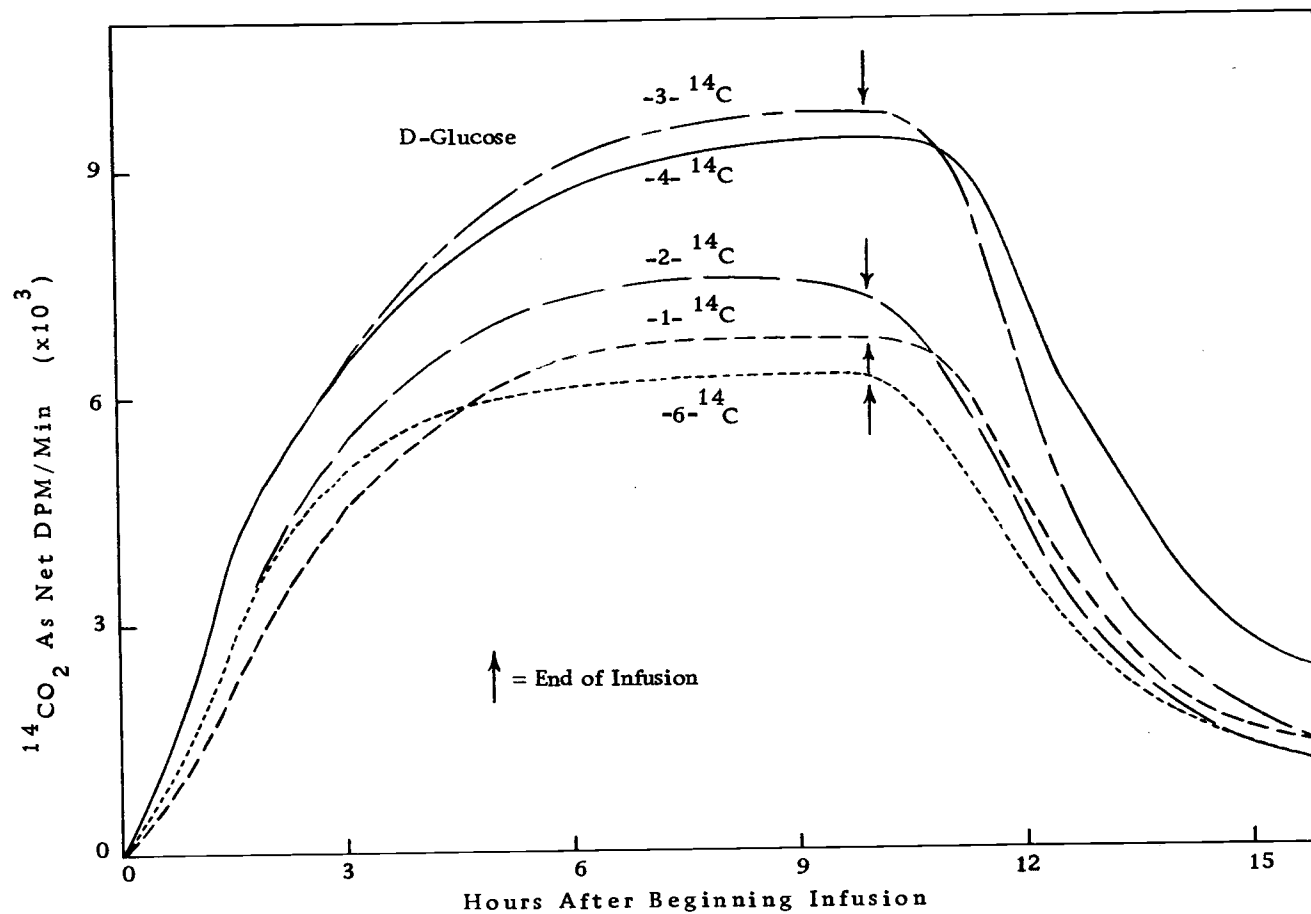


Figure 5. Rates of $^{14}\text{CO}_2$ production from rates utilizing ^{14}C -specifically labeled glucose substrates administered by continuous intravenous infusion.

Table II. Integrated Yields of Glucose Radioactivity in Respiratory CO₂

Labeled carbon atoms of glucose	Integrated yields ¹ of ¹⁴ C in respiratory CO ₂
1	46
2	53
3	70
3, 4	69
4 ²	68
6	43

¹ Labelled glucose was administered into rats by continuous infusion for a period of 10 hours and the measurement of respiratory ¹⁴CO₂ was continued for another 5 hours. Yield of respiratory ¹⁴CO₂ at 15 hours after beginning of substrate infusion, expressed as percentage of total ¹⁴C radioactivity, of the labelled carbon atom of glucose administered during a 10 hour period.

² Yield of ¹⁴CO₂ from C-4 of glucose is obtained by difference making use of data obtained in the glucose-3-¹⁴C and glucose-3(4)-¹⁴C experiments.

slope was observed throughout the first 10 hours. It is difficult to attach any significance to the latter observation, particularly in view of the fact that data for C-4 of glucose were obtained by difference making use of data obtained in glucose-3(4)- ^{14}C and glucose-3- ^{14}C experiments. It is noted that upon termination of substrate infusion, rates for the conversion of carbon atoms to respiratory CO_2 declined sharply and reached insignificant levels 5 hours after substrate infusion. This fact implied that a fraction of infused glucose may have been incorporated into endogenous reserve and hence was not catabolized immediately.

Several conclusions, qualitative in nature, with regard to glucose pathways in rats can be drawn from an analysis of the data given in Figure 5 and Table II. The fact that C-3 or C-4 of glucose was converted to respiratory CO_2 at a higher rate and to a larger extent than any other carbon atoms of glucose points to the predominant role played by the EMP-pyruvate decarboxylation pathway in rats (33)(Figure 1). The fact that rates of CO_2 production are approximately the same with these two carbon atoms indicates that: (1) two trioses derived from glucose via the EMP pathway are in rapid equilibrium with each other and hence they display practically identical catabolic behaviors, and (2) C-3 and C-4 of glucose were converted to respiratory CO_2 primarily via the EMP-pyruvate decarboxylation pathway since the fates of these two carbon atoms via other pathways

such as the PP pathway and the GA pathway are quite different from each other and would show up as significant differences in rates and extent of conversion to respiratory CO_2 .

The rate of the conversion of C-2 of glucose to CO_2 is found to be only slightly greater than that of either C-1 or C-6. If glucose is catabolized exclusively via the EMP-TCA pathway (Figure 1), acetyl CoA derived from glucose is likely to interact with endogenous compounds such as fatty acids, amino acids, etc. prior to its oxidation to CO_2 . Such a situation will result in a preferential conversion of the carboxyl carbon atoms (equivalent to C-1 and C-6 of glucose) to respiratory CO_2 , by a substantial factor, over that of the methyl carbon atoms (equivalent to C-2 and C-5 of glucose) (28). Consequently, the findings that the rates for C-1 and C-6 of glucose to CO_2 are only slightly lower than that of C-2 in magnitudes provide one with indications that pathways other than the EMP route occur in rats. Previously, it has been reported by many workers (9, 15, 20, 21, 33, 38) that the PP pathway and the GA pathway are operative in rats.

Determination of Pathway Participation

Use can also be made of the data or rates of $^{14}\text{CO}_2$ production given in Figure 5 to devise a method for pathway estimation on the basis of catabolic rates of individual pathways. Such a method can be developed since the observed rates of $^{14}\text{CO}_2$ production from certain

carbon atoms of glucose are closely related to the rates of individual glucose pathways. In devising a method to estimate the rate of individual catabolic pathways of glucose, several basic assumptions are required. These assumptions are:

- (1) There are three concurrent pathways for glucose catabolism in intact rats. These pathways are the EMP-TCA pathway, the PP pathway and the GA pathway (Figure 1).
- (2) There is no significant drainage of intermediates associated with the GA pathway, particularly the glucuronides, ascorbic acid, and other intact 6-carbon intermediates.
- (3) The trioses derived from glucose via the EMP pathway are in rapid equilibrium with each other and hence they have identical catabolic behaviors.
- (4) There is no significant drainage of pyruvate, derived from glucose, in the biosynthetic pathways. In other words, pyruvate, once formed, is promptly decarboxylated oxidatively to yield CO_2 and acetyl CoA.
- (5) CO_2 fixation of the $\text{C}_3 + \text{C}_1$ type is not an important process in the over-all rat metabolism.

Verification of each of the foregoing assumptions has been provided in either the present work or from findings in other laboratories.

Let,

G_{cat} = rate of glucose catabolism.

R_3 (or R_4) = rate of $^{14}CO_2$ production in dpm/min. with rats utilizing infused glucose-3- ^{14}C (or glucose-4- ^{14}C) at the metabolic steady state.

It is recognized that R_3 (or R_4) is made up of

three components; namely, R_{3e} (or R_{4e}) =

rate of $^{14}CO_2$ production from infused glucose-3- ^{14}C (or glucose-4- ^{14}C) at the metabolic

steady state via the EMP-TCA pathway

(equivalent to the EMP-pyruvate decarboxylation

pathway); R_{3p} (or R_{4p}) = rate of $^{14}CO_2$ produc-

tion from infused glucose-3- ^{14}C (or glucose-

4- ^{14}C) at the metabolic steady state via the PP

pathway; and R_{3n} (or R_{4n}) = rate of $^{14}CO_2$

production from infused glucose-3- ^{14}C (or

glucose-4- ^{14}C) at the metabolic steady state

via the GA pathway. Hence, $R_3 = R_{3e} + R_{3p} +$

R_{3n} and $R_4 = R_{4e} + R_{4p} + R_{4n}$.

R_1 = rate of $^{14}CO_2$ production in dpm/min. with rats utilizing infused glucose-1- ^{14}C at the metabolic

steady state. It is recognized that R_1 is made

up of three components; namely, R_{1e} = rate of

$^{14}\text{CO}_2$ production from infused glucose-1- ^{14}C at the metabolic steady state via the EMP-TCA pathway; R_{1p} = rate of $^{14}\text{CO}_2$ production from infused glucose-1- ^{14}C at the metabolic steady state via the PP pathway; and R_{1n} = rate of $^{14}\text{CO}_2$ production from infused glucose-1- ^{14}C at the metabolic steady state via the GA pathway. Hence, $R_1 = R_{1e} + R_{1p} + R_{1n}$.

R_2 = rate of $^{14}\text{CO}_2$ production in dpm/min. with rats utilizing infused glucose-2- ^{14}C at the metabolic steady state. It is recognized that R_2 is made up of three components; namely, R_{2e} = rate of $^{14}\text{CO}_2$ production from infused glucose-2- ^{14}C at the metabolic steady state via the EMP-TCA pathway; R_{2p} = rate of $^{14}\text{CO}_2$ production from infused glucose-2- ^{14}C at the metabolic steady state via the PP pathway; and R_{2n} = rate of $^{14}\text{CO}_2$ production from infused glucose-2- ^{14}C at the metabolic steady state via the GA pathway. Hence, $R_2 = R_{2e} + R_{2n} + R_{2p}$.

R_6 = rate of $^{14}\text{CO}_2$ production in dpm/min. with rats utilizing infused glucose-6- ^{14}C at the metabolic steady state. It is recognized that R_6 is

made up of three components; namely, R_{6e} = rate of $^{14}\text{CO}_2$ production from infused glucose-6- ^{14}C at the metabolic steady state via the EMP-TCA pathway; R_{6p} = rate of $^{14}\text{CO}_2$ production from infused glucose-6- ^{14}C at the metabolic steady state via the PP pathway; and R_{6n} = rate of $^{14}\text{CO}_2$ production from infused glucose-6- ^{14}C at the metabolic steady state via the GA pathway. Hence, $R_6 = R_{6e} + R_{6p} + R_{6n}$.

S = specific activity of the infused glucose specifically labeled at a given carbon atom at the metabolic steady state, expressed in dpm/mg.

G_{er} = catabolic rate of the infused glucose via the EMP-TCA pathway at the metabolic steady state, expressed in mg/min.

G_{pr} = catabolic rate of the infused glucose via the PP pathway at the metabolic steady state, expressed in mg/min.

G_{nr} = catabolic rate of the infused glucose via the GA pathway at the metabolic steady state, expressed in mg/min.

G_e = fraction of the catabolized glucose, at the metabolic steady state, via the EMP-TCA

pathway, expressed as percentage of catabolized glucose.

G_p = fraction of the catabolized glucose, at the metabolic steady state, via the PP pathway, expressed as percentage of catabolized glucose.

G_n = fraction of the catabolized glucose, at the metabolic steady state, via the GA pathway, expressed as percentage of catabolized glucose.

On the basis of present understanding, for each mole of glucose catabolized via the EMP-TCA pathway, the PP pathway or the GA pathway, one mole of CO_2 will be produced from C-3 (or C-4), C-1 and C-6 of glucose, respectively. The catabolic rate of the individual glucose pathways can be calculated making use of the specific activity of the infused glucose expressed as dpm/mg as shown in Table I. It is understood that the specific activity of blood glucose at the metabolic steady state is found to be the same as that of the infused glucose.

Thus, the catabolic rate of the EMP-TCA pathway can be expressed as:

$$G_{er} = \frac{R_{3e}}{S} = \frac{R_{4e}}{S} \quad (1)$$

The catabolic rate of the PP pathway can be expressed as:

$$G_{pr} = \frac{R_{1p}}{S} \quad (2)$$

The catabolic rate of the GA pathway can be expressed as:

$$G_{nr} = \frac{R_{6n}}{S} \quad (3)$$

and the over-all rate of glucose catabolism is,

$$G_{cat} = G_{er} + G_{pr} + G_{nr} \quad (4)$$

Since it has been assumed that there are only three concurrent catabolic pathways operating in rats, it follows that relative participation or contribution of individual catabolic pathways in over-all glucose catabolism can be expressed as:

$$G_e = \frac{G_{er} \times 100}{G_{cat}} \quad (5)$$

$$G_p = \frac{G_{pr} \times 100}{G_{cat}} \quad (6)$$

$$G_n = \frac{G_{nr} \times 100}{G_{cat}} \quad (7)$$

The exact values for the terms R_{3e} , R_{1p} and R_{6n} needed for accurate pathway estimation cannot be readily ascertained from the observed data on R_3 , R_1 and R_6 since the latter terms are each made up of three components and some of the components cannot be directly measured. However, the magnitudes of the terms R_{3e} , R_{1p} and R_{6n} can be crudely estimated thereby making it possible to make a crude estimation of pathway participations. Thus, the conversion of C-3 of glucose to respiratory CO_2 via either the PC pathway or the GA pathway involves various steps and intermediates via long catabolic

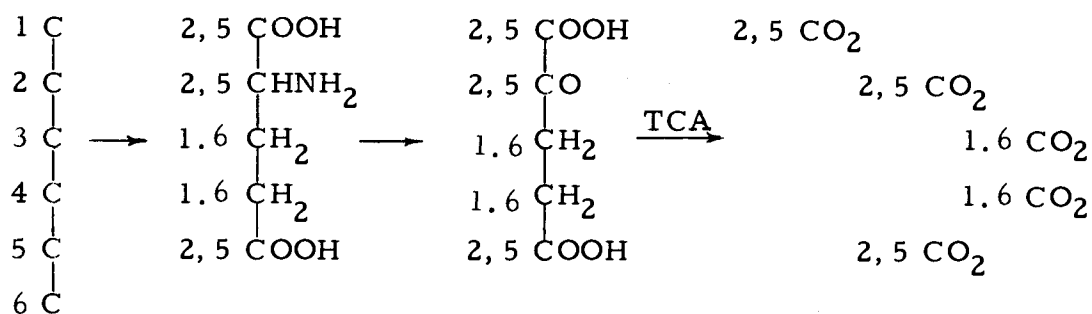
sequences (Figure 1). Participation of these intermediates in biosynthetic and exchange reactions will much reduce the opportunity for C-3 of glucose to be converted to respiratory CO_2 . Consequently, unless there are other evidences indicating that the PC and the GA are predominant pathways in glucose catabolism, one can consider as a close approximation that $R_3 = R_{3e}$.

In order to estimate the approximate values of R_{1p} and R_{6n} , one needs to first examine the makeup of the terms R_1 and R_6 . In the case of R_1 , of the three component terms; viz. R_{1e} , R_{1p} and R_{1n} , R_{1n} is likely to be relatively small in magnitude since the conversion of C-1 of glucose via the GA pathway also involves a long catabolic sequence subject to dilution of various endogenous intermediates. In contrast, conversion of C-1 of glucose via the PP pathway is direct and hence R_{1p} is a major component of R_1 even when the participation of the PP pathway is limited in extent. As an approximation one can therefore consider that $R_1 = R_{1e} + R_{1p}$. Similarly, in the case of R_6 , of the three component terms; viz. R_{6e} , R_{6p} and R_{6n} , R_{6p} is likely to be relatively small in magnitude since the conversion of C-6 of glucose via the PP pathway involves a long catabolic sequence subject to dilution of various endogenous intermediates, while conversion of C-6 to CO_2 via the GA pathway is a more direct process. It follows that as an approximation $R_6 = R_{6e} + R_{6n}$. With the foregoing considerations in mind, if one can devise a means to estimate the

values of R_{1e} and R_{6e} , one should be able to have a crude estimation of the values of R_{1p} and R_{6n} , needed in calculating the rates of the PP and the GA pathways.

Information on the net rate for the conversion of either C-1 (R_{1e}) or C-6 (R_{6e}) of glucose to CO_2 via exclusively the EMP-TCA pathway, cannot be directly obtained from the radiorespirometric data collected in the glucose experiments. However, such information can be acquired in experiments using reference compounds simulating a key catabolic intermediate of glucose in the EMP pathway (5, 14). Examination of possible compounds that can be used as a reference compound revealed that glutamic acid is the best choice.

The use of glutamate as a reference compound is derived from the considerations that glutamate is known to be present in small amounts in the circulatory system of rats and the chemical nature of glutamate is such that continuous infusion of a trace amount into the circulatory system would not incur any detrimental effect. The biogenic relationship between the carbon skeleton of glutamate and glucose is well established as illustrated in the following:



D-glucose L-glutamate

Numerals designate positions of carbon atoms in glucose.

It is visualized that the conversion of C-2 of glucose to respiratory CO₂ is primarily by way of the EMP-TCA sequence. Hence one can consider that $R_2 = R_{2e}$ and use can be made of the observed values of R_2 as a reference value to calculate the values of R_{1e} and R_{6e} .

In order to obtain information on the relative rates for the conversion of various carbon atoms of glutamic acid, a series of radiorespirometric experiments were carried out using DL-glutamic acid-1, -2, and -3(4)-¹⁴C as individual substrates. The conditions used for the glutamate experiments are the same as that for the glucose experiments. Unlabeled glucose was infused into the rat at a rate of 150 mg/hr., along with trace amount of ¹⁴C-specifically labeled glutamate. DL-glutamic acid was used instead of L-glutamic acid, since all the ¹⁴C-labeled glutamic acids were synthesized chemically and hence they are racemic mixtures. The findings in this series of experiments are given in Figure 6.

It is noted that the rate for the conversion of labeled carbon

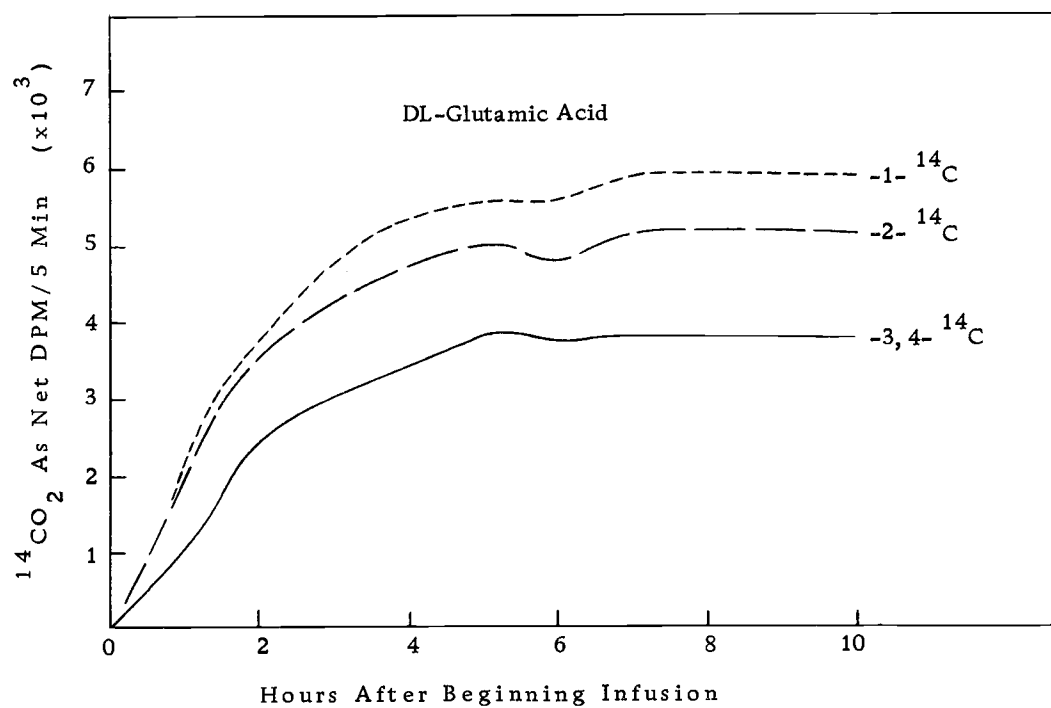


Figure 6. Rates of $^{14}\text{CO}_2$ production from rats utilizing ^{14}C -specifically labeled DL-glutamic acid administered by continuous intravenous infusion. Unlabeled glucose was infused as co-substrate at a rate of 150 mg/hr.

atoms of DL-glutamate to respiratory CO_2 reached a constant level a few hours after the beginning of the substrate infusion. It is therefore reasonable to believe that a metabolic steady state in rats can be realized with respect to the infused glutamate. It is further noted that C-2 of glutamate was converted to respiratory CO_2 at a rate considerably greater than that of C-3(4). Using the rate data collected at 8 hours after the beginning of infusion, the ratio:

$$\frac{\text{rate of } \text{CO}_2 \text{ from C-2 of glutamate}}{\text{rate of } \text{CO}_2 \text{ from C-3(4) of glutamate}} \text{ is } \frac{5.5 \times 10^3 \text{ dpm/5 min.}}{3.8 \times 10^3 \text{ dpm/5 min.}} = 1.37$$

Taking into consideration the biogenic relationships between glutamate and glucose; i. e., $\frac{\text{rate of } \text{CO}_2 \text{ from C-2 of glutamate}}{\text{rate of } \text{CO}_2 \text{ from C-3(4) of glutamate}} =$

$$\frac{\text{rate of } \text{CO}_2 \text{ from C-2 of glucose via EMP-TCA cycle pathway}}{\text{rate of } \text{CO}_2 \text{ from C-1 or C-6 of glucose via EMP-TCA cycle pathway}},$$

if the infused glucose were catabolized exclusively via the EMP-TCA pathway, one would expect that the C-2 of glucose will be converted to CO_2 at a rate 1.37 fold of that of C-1 or C-6. It follows that

$$R_{1e} = \frac{R_2}{1.37} \quad (8)$$

and

$$R_{1p} = R_1 - R_{1e} = R_1 - \frac{R_2}{1.37} \quad (9)$$

Similarly, the rate for the conversion of C-6 of glucose to respiratory CO_2 via the EMP-TCA pathway can be expressed as

$$R_{6e} = \frac{R_2}{1.37} \quad (10)$$

and

$$R_{6n} = R_6 - R_{6e} = R_6 - \frac{R_2}{1.37} \quad (11)$$

Applying the foregoing equations for the estimation of catabolic rates and participation of individual pathways and using the radio-respirometric data obtained in the present experiment, the results are summarized in Table III. In the present work, glucose was infused into the rat at a rate of 2.50 mg/min. On the basis of the data given in Table III, one finds that of the infused glucose, 2.05 mg was channeled into catabolic processes. It follows that of the infused glucose, 2.50 mg - 2.05 mg = 0.45 mg or 18% remained uncatabolized either as intact glucose or some other form of anabolic reserve such as glycogen. Such a conclusion is in line with the observed radiorespirometric pattern shown in Figure 5. Thus, upon termination of substrate administration at 10 hours after the beginning of infusion, a considerable amount of substrate radio-activity appeared in the respiratory CO_2 at a declining rate for a period of 5 hours. The integrated yields of respiratory $^{14}\text{CO}_2$ from labeled glucose during the depletion phase (from termination of infusion to the end of the experiment) can be crudely estimated by a visual inspection of the rate data given in Figure 5. Thus, the area under the rate curve obtained in the glucose-3- ^{14}C experiment during the period from 10 hours to 15 hours is approximately one-fifth (or 20%) of the area under the entire curve which is of the same

Table III. Estimation of Relative Participation of Catabolic Pathways of Glucose Using Radiorespirometric Data Obtained in Infusion Experiments.

Data Collected In Radiorespirometric Experiments		Catabolic Rates of Glucose Pathways	
R_2 , dpm/min.	1.52×10^3	The EMP Pathway:	
R_3 , dpm/min.	1.94×10^3	$G_{er} = \frac{R_{3e}}{S}$, mg/min.	1.70
S , dpm/mg	1.14×10^3	(Equation 1)	
R_1 , dpm/min.	1.36×10^3	The PP Pathway:	
R_{1e} , dpm/min. (Eq. 8)	1.11×10^3	$G_{pr} = \frac{R_{1p}}{S}$, mg/min.	0.22
R_{1p} , dpm/min. (Eq. 9)	0.25×10^3	(Equation 2)	
R_6 , dpm/min.	1.26×10^3	The GA Pathway:	
R_{6e} , dpm/min. (Eq. 10)	1.11×10^3	$G_{nr} = \frac{R_{6n}}{S}$, mg/min.	0.13
R_{6n} , dpm/min. (Eq. 11)	0.15×10^3	(Equation 3)	
Rate of infusion, mg/min.		Total Catabolism: G_{cat} , mg/min.	2.05
		(Equation 4)	
Estimated Relative Participation of Pathways			
Participation of the EMP pathway $G_e = \frac{G_{er} \times 100}{G_{cat}} = \frac{1.70 \times 100}{2.05} = 83\%$			
Participation of the PP pathway $G_p = \frac{G_{pr} \times 100}{G_{cat}} = \frac{0.22 \times 100}{2.05} = 11\%$			
Participation of the GA pathway $G_n = \frac{G_{nr} \times 100}{G_{cat}} = \frac{0.13 \times 100}{2.05} = 6\%$			

magnitude as the calculated value; i. e., 18% for anabolized glucose. This fact renders support to the validity of several assumptions made in the analysis of the present radiorespirometric data.

As indicated by the results given in Table III, the EMP pathway is by far the predominant route for glucose catabolism in intact rats. The estimated contribution of the PP pathway amounting to 11% of catabolized glucose is equivalent to 4% expressed in terms of contribution of the pentose cycle pathway (17, 32). Previously, Landau and Katz (15) in studies with adipose tissue, have reported that the contribution of the pentose cycle pathway to glucose catabolism is 10 to 15%. It is possible that the pentose cycle is playing a more important role in adipose tissue inasmuch as the major byproduct of the pentose cycle pathway; i. e., NADPH, is needed for the synthesis of fatty acids, a major function of the adipose tissue. The GA pathway is estimated to play a very minor role (approximately 6%) in glucose catabolism. Earlier, Landau et al. (20) reported that with rat epididymal tissue, the GA pathway contributes at most 10% in over-all glucose catabolism.

It is also of interest to analyze the information on relative participation of various glucose pathways taking into consideration both the catabolic and anabolic routes. When the pathway data are expressed on the basis of total administered glucose and considering that the portion of glucose which was not engaged immediately in

catabolic processes, was engaged in anabolic functions, one can express the pathway information obtained in the present work as follows:

Total amount of glucose administered to the rat per minute

$$= 2.50 \text{ mg or } 100\%$$

Amount of glucose engaged in anabolic processes per minute

$$= 0.45 \text{ mg or } 18\%$$

Amount of glucose engaged in catabolic processes per minute

$$= 2.05 \text{ mg or } 82\%$$

Amount of glucose routed into the EMP pathway per minute

$$= 1.70 \text{ mg or } 68\%$$

Amount of glucose routed into the PP pathway per minute

$$= .22 \text{ mg or } 9\%$$

Amount of glucose routed into the GA pathway per minute

$$= .13 \text{ mg or } 5\%$$

Estimated relative participation of individual catabolic pathways in over-all glucose catabolism (Table III) represents upper limits of contribution of each catabolic route. This is true since in calculating the values of R_{3e} , R_{1p} and R_{6n} , the contributions of indirect routes for the conversion of some glucose carbon atoms to respiratory CO_2 were ignored. However, it is noted that the estimated total amount of glucose engaged in prompt catabolism is 18% of the infused glucose, a value of the same magnitudes as that concluded from the observed

radiorespirometric pattern (Figures 3 and 4). In the latter regard, approximately 20% of the infused glucose was found to be converted to respiratory CO_2 during the depletion phase. One is therefore inclined to believe that the pathway information obtained in the present work is not too far from the true catabolic behavior of the rat.

It can therefore be concluded that when glucose is infused into rats at post-absorptive state, a major fraction is routed into catabolic functions with the EMP-TCA being the most important catabolic pathway. The PP pathway and the GA pathway occur in intact rats but are not important catabolic mechanisms. It should be noted that in the present work, the contribution of the EMP pathway in glucose catabolism is estimated by direct means. This is in contrast to previously reported methods in which only the contribution of the PP pathway is measured directly and the EMP pathway is estimated as the difference between total glucose utilized and the fraction of glucose routed into the PP pathway. These earlier approaches assumed that the administered glucose is engaged completely in catabolic functions.

The present work provides one with information on the rate of catabolism via a given pathway at metabolic steady state. In fact, the use of infusion techniques and associated methodology described herein demonstrated that rate of a catabolic event in mammals can be directly measured. The availability of rate information is believed

of great importance in studying the effect of internal or external factors upon carbohydrate metabolism in mammals.

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