

Effects of the Concentration of Medium Glucose on Glucose Disappearance and Oxidative Metabolism by Liver Slices of Rats*

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The concept that enzymes act by forming an intermediate enzyme-substrate complex in the metabolic process is well known. A half century ago, Henri¹⁾ and Brown²⁾ carried out experiments designed to study the effect of the sucrose concentration on the activity of sucrase. In general, the effect was diphasic; the low concentrations of substrate had a marked effect, but the higher concentrations had practically no effect, on the reaction velocity. Henri interpreted the diphasic nature of this effect to mean that the enzyme formed an intermediate complex with its substrate, and that reaction could not take place without the formation of this intermediate complex. All subsequent works have confirmed this view³⁾.

General considerations on the rate of reaction in enzyme chemistry are adequately described by the work of Van Slyke⁴⁾. At low substrate concentrations two factors control the rate of reaction; a) the intrinsic nature of the enzyme itself on catalyzing the reaction, and b) the frequency of collision of the enzyme with its substrate. The second factor will be directly proportional to the concentration of substrate, and the rate of reaction will proceed according to the first order kinetics.

As the concentration of substrate is increased, the collision frequency no longer becomes a factor in the enzyme reaction. At this point, the rate will be governed by zero order kinetics and the rate limiting step is the enzymatic reaction proper.

The first satisfactory mathematical analysis of the diphasic activity curve was carried out by Michaelis-

Menten⁵⁾ and by Van Slyke et al⁶⁾. Michaelis and Menten assumed that intermediate substrate-enzyme complex was reversibly formed in accordance to the mass law, and believed that the rate of breakdown of enzyme-substrate complex to form the product was small compared to the rate of establishment of the equilibrium in the reaction forming enzyme-substrate complex from the substrate and enzyme.

The Michaelis-Menten concept of enzyme-substrate combination has been of great usefulness in enzymology. While it is easy to demonstrate the effect of substrate concentration upon enzyme reaction as in the metabolic processes of tissues, no one has conclusively shown how these reactions via the enzyme-substrate complexes are brought about. It is obvious that enzymes are concerned at every step of reactions in tissue metabolism.

When one substrate breaks down into some final products in the course of tissue metabolism, the reaction does not necessarily proceed in a single step; the actual metabolic pathway may be a complicated multi-step process. For example, as glucose is oxidized into respiratory CO₂ in the tissues, glucose undergoes many orderly sequences of reactions, which are called glycolysis and Krebs cycle⁷⁾ and in the course of such a metabolic pathway, CO₂ is released by oxidative decarboxylation from various intermediate metabolites.

If one assumes that metabolic process of glucose oxidation to CO₂ proceeds in a steady state, where the rate of enzymatic reactions at every step is constant, the overall rate of conversion of glucose into CO₂ must be constant. Conditions for metabolic regulation must involve concentration of substrate,

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intrinsic factors of metabolic machinery in the tissue cell and the action of various compounds as inhibitors or activators on enzymes. Of the conditions described above, if constant concentrations of multienzyme system and various enzyme effectors for the given substrate are maintained, the rate of reaction should be governed by the concentration of the substrate surrounding metabolic pool according to the mass law. Since enzymatic machinery in the living organism does not allow equilibrium with a sole substrate pool and since these various substrates compete with the same enzymatic system each other in the metabolism of living body, it is impossible to measure the maximum capacity of metabolizing the given substrate by the tissue enzymatic machinery in the intact living animal. We are, therefore, interested in determining the maximum capacity of metabolizing a single substrate by the enzymatic machinery of the tissue, especially for glucose which is known as the most important energy source in the living body.

The method described here for studying the kinetics of conversion of C^{14} -glucose to CO_2 by tissue slices involved a procedure in which the incubation medium, having various concentrations of C^{14} -glucose and the gas phase, was periodically separated from the slices and replaced with a fresh medium and gas so as to maintain the constant circumstances.

This procedure enabled us to determine the amount and specific activity (SA) of the CO_2 produced by the slices in several successive periods during the course of experiment. It also had the advantage of presenting the tissue with a substrate of essentially constant concentration and SA and of prolonging the life of tissue slices by removing harmful metabolic products. Liver slices have been maintained in active metabolic state for as long as 8 hours under the conditions described here. Application of this method enabled us to determine the maximum capacity of oxidative metabolism of exogenous C^{14} -glucose by the liver slices in the steady state and to evaluate the kinetic characteristics of the tissue enzymatic system oxidizing the medium glucose.

Method

Liver slices of Sprague-Dawley strain rats, ranging in weight from 250gm to 300gm, were used. Weight of tissue slices incubated in every experiment ranged

from 1.5 to 2.5grams.

General procedure: Liver slices were incubated in 50cc syringes with incubation mixture consisting of 15cc of phosphate buffer⁸⁾ to which were added varying amount of C^{14} -glucose and 15cc of CO_2 free 100% oxygen. The syringes were capped airtight with 2 way stop cock and shaken to-and-fro in the water bath maintained at 38°C. Such a shaking in the water bath ensured thorough mixing and rapid renewal of the interface between the gas and liquid, with minimum disruption of the slices. During the course of 7-8 hours of incubation period, incubation mixtures were replaced hourly with fresh C^{14} -glucose medium and gas in order to equilibrate the exogenous C^{14} -glucose medium and tissue metabolic pool. On the other hand, hourly samples of incubation medium and gases, obtained while replacing the incubation mixture, were anaerobically transferred into a specially prepared "sample transfer assembly"^{9,10)}. Transfer assembly was made of a outer tube and a inner conical tube into which were placed about 3ml of CO_2 free 2N-NaOH solution and then the assembly tubes were evacuated to a negative pressure of 30psi. After incubation mixtures were transferred into the assembly tube, metabolic CO_2 derived from liver slices in the gas sample was trapped by an alkaline solution placed in an inner conical tube of the transfer assembly and the alkaline carbonate sample was obtained. These alkaline carbonate samples were quantitatively precipitated with 0.3N $BaCl_2$ solution into $BaCO_3$. Thereafter the radioactivity of $BaCO_3$ was counted with an endwindow Geiger-Müller counter as an infinitely thin sample by correcting the self absorption. The weight of the $BaCO_3$ plated is a measure of the total CO_2 production by the liver slices. While liquid C^{14} -glucose medium in the transfer assembly was analyzed for concentration and SA of glucose. Glucose levels of incubation medium were determined by method of Somogyi¹¹⁾ and Nelson¹²⁾ and SA of medium glucose by that of wet combustion described by Van Slyke and Folch¹³⁾.

Calculation: Glucose disappearance rate from the liver slices were determined by the concentration difference of glucose before and after the incubation and volume of incubation medium.

Total CO_2 production rates were calculated from the weight of $BaCO_3$ precipitate obtained from the

Table 1. Glucose disappearance rate and conversion of glucose into respiratory CO₂

medium conc. of glucose (mg%)	number of cases	glucose mg/hr/gm	disapp. rate μ M/hr/gm	total CO ₂ production rate μ M/hr/gm	RSA %	CO ₂ from glucose μ M/hr/gm	amt. of glucose into CO ₂ μ M/hr/gm	% of glucose disapp. into CO ₂ %
30	5	2.2 ±.54	12.2 ±3.92	54.3 ±1.63	27.1 ±1.32	14.9 ±1.04	2.48 ±.17	20.0 ±4.51
40	4	4.71 ±.7	26.2 ±5.1	64.4 ±7.47	39.2 ±9.54	25.2 ±9.19	4.2 ±2.0	16.0 ±12.3
90	4	8.35 ±2.32	46.4 ±7.39	77.9 ±5.24	48.6 ±10.4	37.9 ±14.8	6.3 ±2.46	13.6 ±5.1
120	9	10.86 ±2.92	60.3 ±17.5	84.8 ±22.5	66.4 ±15.3	56.3 ±30.8	9.38 ±5.12	15.6 ±6.8
170	8	10.94 ±2.27	60.8 ±12.7	94.7 ±13.3	64.3 ±4.58	60.9 ±18.8	10.15 ±4.24	16.7 ±3.1
270	1	9.8	54.5	111.2	66.8	74.3	12.4	22.8
380	1	12.3	68.3	110.2	71.7	79.0	13.2	19.3

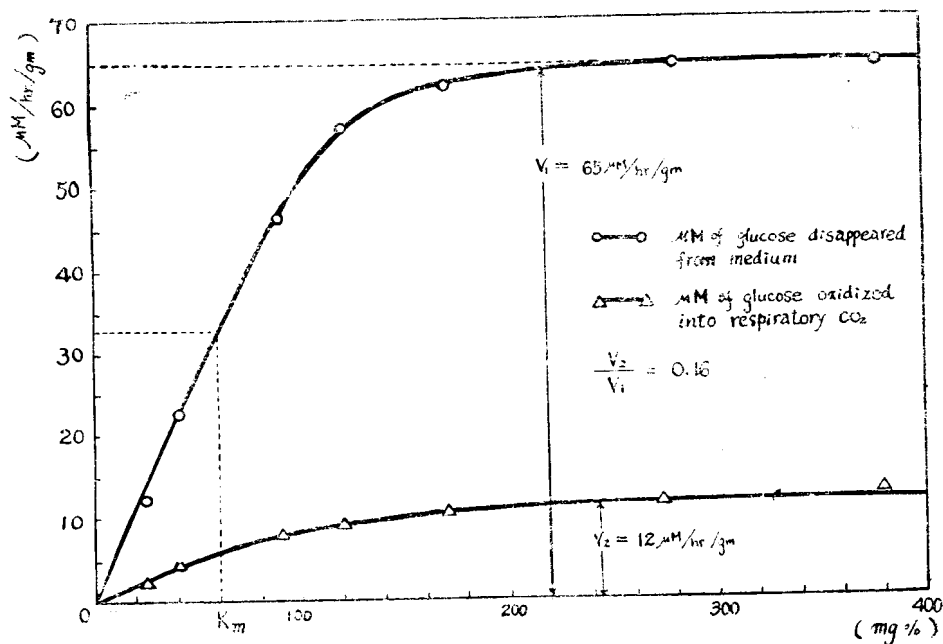


Fig. 1. Glucose Disappearance and Oxidation Rate

inner tube of the transfer assembly.

Relative specific activity (RSA) was obtained from the ratio of SA of CO₂ to SA of medium glucose. This gave the fraction of the metabolic CO₂ derived from the added C¹⁴-glucose. Relative glucose disappearance (RGD) was calculated as follows:

$$RGD = \frac{\text{total CO}_2 \text{ production rate} \times \text{RSA}}{\text{glucose disappearance rate} \times 6}$$

This gave the fraction of glucose disappeared from the incubation medium into metabolic CO₂.

Results

Liver slices weighing 1-2gm were incubated with various concentrations of C¹⁴-glucose as described

above. The metabolic activities of each slice incubated showed the characteristic figures during the time course of experiments. The total CO₂ production rates were initially high, then fell rapidly and, after about 3-4 hours, showed relatively constant values, while glucose disappearance rates from the medium C¹⁴-glucose and relative specific activity rose during the first 4 hours and remained approximately constant throughout the experimental period. The initial unsteady metabolic activities participate in the equilibration processes labeled substrate carbon with the metabolic pool of cell interior.

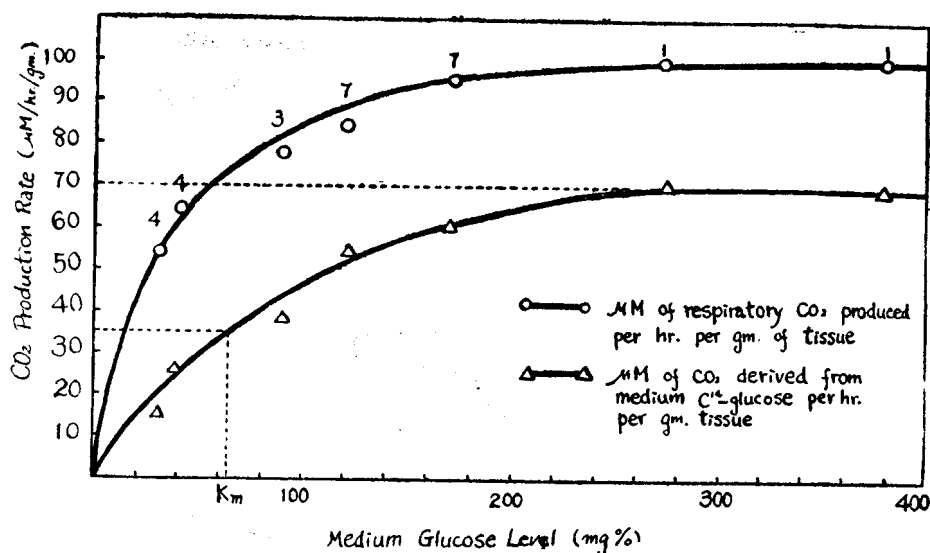


Fig. 2. Total CO₂ Production Rate and CO₂ derived from Exogenous C¹⁴-glucose

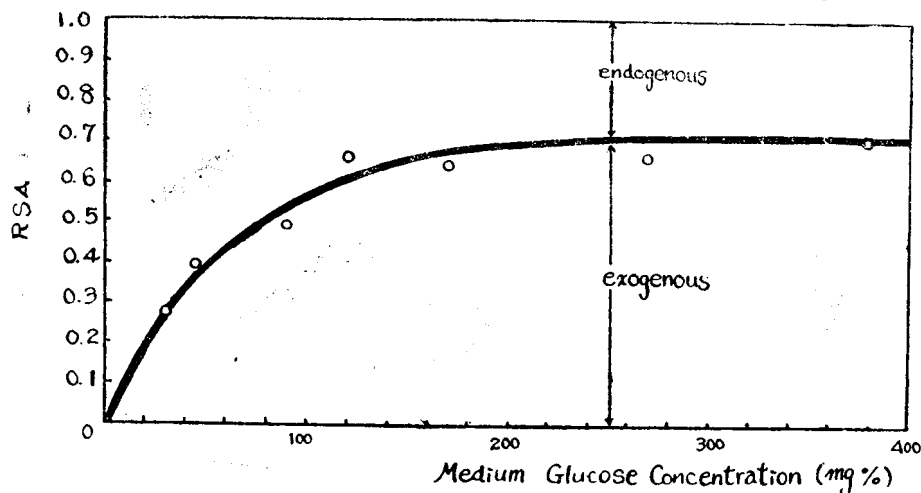


Fig. 3. Correlation between RSA and Medium Glucose Concentration

At least 3 hours after incubation, the complete equilibration between C¹⁴-glucose medium and intracellular enzymatic system seems to be attained. Therefore, every metabolic rate determined at a given concentration of C¹⁴-glucose was taken from the mean values obtained from hourly samples withdrawn 3 hours after the onset of incubation. The data obtained at the experiments incubated with various concentrations of C¹⁴-glucose are summarized in the Table 1. The glucose disappearance rate from incubation medium rose rapidly at the low concentration of C¹⁴-glucose and appeared to approach a plateau at the C¹⁴-glucose concentration of 150mg% and then to slope down to zero at the concentrations above 150mg%, as shown in the Figure 1. The mean maximum value attained was about 65μM/hr/gm of tissue. The total CO₂ production rate was initially

low, then increased rapidly as concentrations of C¹⁴-glucose in incubation mixtures increased upto 150mg %, thereafter steady maximum value of about 100 μM/hr/gm was attained(Fig. 2).

The contour of the relative specific activity vs. concentration of C¹⁴-glucose curve was also same as that of the glucose disappearance vs. the total CO₂ production rate, and the maximum value attained was about 70%(Fig. 3). This represents the maximum capacity of enzymatic system of liver slices oxidizing the medium C¹⁴-glucose into respiratory CO₂.

The CO₂ production rate derived from C¹⁴-glucose was calculated by the total CO₂ production rate and RSA, the maximum value attained at the concentration of C¹⁴-glucose of about 150mg%, and obtained about 70μM/hr/gm(Fig. 2).

As described above, effects of concentration of

C¹⁴-glucose on glucose disappearance and oxidative metabolism of C¹⁴-glucose by the liver slices represent the general kinetic characteristics of enzyme reaction, as shown in the Figures 1, 2 and 3. From the diphasic nature of curves, we obtained Michaelis constant (K_m) of enzyme system of cell membrane which concerned with glucose penetration and of multienzyme system concerned with glucose oxidation into CO₂ by Michailis-Menten analysis. K_m determined by concentration of glucose at half maximum velocity was 60mg% or 3.33×10⁻⁴M/L for the enzyme concerned with glucose penetration and 65mg% or 3.62×10⁻⁴M/L for the enzyme group concerned with glucose oxidation in the metabolic pool of the liver slices. There were little changes in the relative glucose disappearance (RGD), which is a fraction of glucose disappeared into CO₂, for all ranges of concentrations of C¹⁴-glucose, since both factors, glucose disappearance rate and CO₂ production rate from glucose, changed proportionately as concentrations of C¹⁴-glucose varied. Mean value of RGD was 17.2%. Therefore 17.2% of glucose disappearance rate was oxidized into respiratory CO₂.

Discussion

The fact that every metabolic activity of liver slices determined in this paper appear to approach a plateau 3 or 4 hours after incubation during the time course of experiment, during which liver slices exposed to a constant concentration and specific activity of C¹⁴-glucose in a incubation medium, reflects the equilibration process of labeled glucose with the tissue metabolic pool as described in our earlier paper¹⁰⁾ and Chaikoff et al⁹⁾. Therefore, the plateau value of each metabolic activity at a given concentration of C¹⁴-glucose in a incubation medium is believed to represent a maximum capacity in a metabolic process of liver slices under steady state equilibrated with given concentration of C¹⁴-glucose.

The experimental approach described here makes it possible to study the kinetics study of metabolic activity of liver slices in the metabolic process of C¹⁴-glucose under a steady state.

The glucose disappearance rate is increased at low concentrations of C¹⁴-glucose but at higher concentrations there was practically no effect on the glucose disappearance rate as illustrated Figure 1. The

diphasic nature of such a curve on the glucose disappearance means that enzymatically controlled processes take place to transport glucose from the incubation medium into cell interior. To confirm this point, the evidence is available for membrane localization. The resolution power, therefore, remains insufficient to enable decision to be made as to whether the enzyme is attached to the cell membrane. However, the recent methods of histochemistry introduced the possibility of localizing enzyme activity with surprising accuracy¹¹⁾. So far this has been possible mainly with regard to alkaline phosphatase, which in the kidney, the intestine and other organs has given very clear and interesting pictures¹⁵⁾.

As in Fig. 1 illustrating the relationship between the glucose disappearance and the concentration curve, 75% of maximum rate was attained at normoglycemic level and the K_m of enzymes concerned with glucose transport at cell membrane was 3.33×10⁻⁴M/L, while K_m for oxidative enzymatic system was 3.62×10⁻⁴M/L as in the process of the exidative metabolism of exogenous C¹⁴-glucose by liver slices. This seems to mean that oxidation of glucose into CO₂ in the tissue metabolic pool did not effect by penetration lag of exogenous C¹⁴-glucose at the cell membrane.

In our experimental approach described here, where the only source of metabolic CO₂ was the glucose added to the medium, the RSA values for CO₂ would approach asymptotically 100% at higher concentration. However, as shown in the Figure 3, the RSA never reached 100% in spite of higher concentration of C¹⁴-glucose and showed only 70% of maximum plateau values. The remaining 30% of total CO₂ production, because it is not derived from the labeled glucose, may appear to be a product of endogenous metabolism. The term endogenous metabolism implies that there is a maximum limit to oxidize the glucose by the enzyme system of metabolic pool or that the pool of endogenous precursors is so large that its turn-over is too slow to be detected during the period of experiments. In our earlier study, the RSA was about 27%¹⁶⁾ in the intact dog and 33%¹⁷⁾ in the isolated beating heart perfused with blood. These values are much lower as compared to that of the incubation experiment described here. The reason for the difference is that

various complex pools of substrates would compete for an enzymatic system of metabolic pools in the blood-perfused at animal or organs. Since single substrate was equilibrated with an enzymatic system of the tissue in this experiment, the value obtained in the latter experiment would be meant to represent maximum capacity of oxidizing the glucose into CO₂.

From the glucose disappearance rate and CO₂ production rate derived from C¹⁴-glucose in the incubation mixture, we can calculate the fraction of glucose disappeared into respiratory CO₂ which is termed here as relative glucose disappearance (RGD). This value was average 19% and independent of medium concentration of glucose. These data were interpreted to mean that a minor part of the glucose disappeared contributes to their oxidative metabolism, though liver slices readily derive a very substantial portion of its oxidative energy from medium glucose.

Summary

Liver slices of Sprague-Dawley rat were incubated with various concentration of C¹⁴-glucose in the incubation medium. A constant condition was maintained by periodic replacement of the incubation medium and gas phase throughout the experimental period, during which hourly samples were withdrawn and analyzed for glucose disappearance rate, and the rate of oxidation of C¹⁴-glucose into CO₂. From 32 cases of experiments in which concentrations of C¹⁴-glucose in the incubation medium were varied, the following results were obtained.

1. Every metabolic rate vs. concentration curve for glucose disappearance rate, relative specific activity and CO₂ production rate from C¹⁴-glucose represents characteristic diphasic nature as in the process of enzyme reaction, and approaches to a plateau at the medium concentration of about 150 mg%.

2. A plateau value for glucose disappearance was 65μM/hr/gm, for RSA, 70% and for CO₂ production rate from C¹⁴-glucose, 70μM/hr/gm. Fractions of glucose disappeared into CO₂ or RGD were average 19% for all ranges of glucose concentrations.

3. Km values for enzyme concerned with glucose transport at the cell membrane and oxidative metabolism were 3.33×10⁻⁴ M/L and 3.62×10⁻⁴M/L respectively. These Km values imply that glucose

oxidation in the metabolic pool would be interfered by penetration lag at the cell membrane.

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국 문 초 록

흰쥐 간 절편에 있어서 용매 C¹⁴-포도당농도가 포도당소실을 및 산화대사과정에 미치는 영향

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Sprague-Dawley 종의 흰쥐 간 절편을 이용하여 용매 C¹⁴-포도당의 농도를 변화시킬때의 용매 C¹⁴-포도당의 소실을 및 산화대사과정을 관찰하였다. Incubation 방법은 매시간마다 새로운 C¹⁴-포도당용매와 가스를 교체함으로써 실험기간중 일정한 외부환경을 유지하고자 노력하였다. 흰쥐 32마리를 7군으로 나누어 각기 다른 농도의 C¹⁴-포도당용매에 incubate 하여 관찰한 결과 다음과 같은 성적을 얻었다.

1) C¹⁴-포도당의 소실을 및 산화대사율의 용매 C¹⁴-포도당농도에 따르는 곡선은 교소반응에서 보는 일탄적곡선을 보이며 용매 C¹⁴-포도당농도 약150mg%에서 평탄선(plateau)을 이루어 최고값을 보였다.

2) 상기곡선의 평탄선에서 얻은 C¹⁴-포도당소실율의 최고값은 65μM/hr/gm, RSA의 최고값은 70%, 용매 C¹⁴-포도당에서 기인된 CO₂ 발생율은 70μM/hr/gm의 최고값을 각각 보였다. 한편 소실된 C¹⁴-포도당이 호흡 CO₂로의 완전산화율(RGD)은 용매 C¹⁴-포도당농도에 좌우되지 않았으며 각종농도에서 비교적 일정한 값 19%를 산출하였다.

3) 포도당의 세포막투과에 관여하는 교소 및 산화대사과정에 관여하는 교소계의 Km을 Michealis-Menten 공식에 의거하여 측정한 결과 전자에서는 3.33×10⁻⁴M/L, 후자에서는 3.62×10⁻⁴M/L을 보였다. 이러한 값은 서로 별차이가 없는것으로 보아 세포내교소계로 인한 C¹⁴-포도당의 산화대사과정은 포도당의 세포막투과치연으로 인한 영향을 받지않음을 지적할수 있다.

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