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# THE KINETICS OF GLUCOSE TRANSPORT INTO CANCER CELLS (KB LINE)

A THESIS

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THE KINETICS OF GLUCOSE TRANSPORT INTO CANCER CELLS (KB LINE)

Approved:

Chairman

Date approved by Chairman: June 1, 1967

To

My loving wife for her moral support.

# ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr. Hong S. Min for his guidance in preparation of this thesis. I would also like to thank Dr. Robert H. Fetner and Dr. Konrad Bachman for their helpful suggestions in preparation of the manuscript and to Mrs. Joyce Williams for her secretarial assistance.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF ILLUSTRATIONS

## SUMMARY

The uptake of glucose by KB cells, a human oral carcinoma grown in tissue culture, was studied to determine the pattern and kinetics of entry.

Glucose -U-$C^{14}$ was used in these experiments and uptake was monitored by measuring the increase in radioactivity of the cell water by liquid scintillation.

The pattern of entry of glucose was very similar to the pattern observed in experiments with other types of cells. The kinetics of transport were enzyme-like and obeyed an adsorption isotherm.

Glucose is believed to enter KB cancer cells by a mobile carrier mechanism and at rates that are similar to those observed in other types of mammalian cells. Carbohydrate transport, therefore, does not seem to play a limiting role in the metabolic activities of the KB cancer cell.

# CHAPTER I

## INTRODUCTION

Materials may be transported into cells by simple diffusion, facilitated diffusion, active transport or pinocytosis.

Simple diffusion across the cell membrane is described by Fick's Law, which states that the rate of diffusion is proportional to the concentration difference between the two phases. The energy for this process is derived solely from the kinetic energy of thermal agitation of the solute molecules. Such diffusion across a cell membrane is affected only by concentration difference, molecular size of the diffusing species, the lipoid solubility of the solute and the magnitude of the surface area available for diffusion (Giese, 1963). However, in biological systems this type of transport is found to be the exception.

A phenomenon frequently observed is mediated or facilitated diffusion. The distinguishing characteristics of this form of diffusion are: substrate specificity, competitive inhibition, saturation kinetics, and Michaelis-Menten kinetics. These characteristics imply that a reaction with a chemical site or structure that is present in only limited amounts occurs in the transport process. This proposed structure or group is said to mediate the transport (Christensen, 1962). The kinetics of the transport follow an adsorption isotherm which suggests that the substrate may adsorb reversibly to a limited number of sites or carriers (Maio, 1962). According to the carrier theory the substrate forms a

complex with a membrane constituent; the complex dissociates on the opposite side of the membrane and returns to the original position where it may again engage additional molecules (Cirillo, 1961; Maio, 1962).

The characteristics of active transport are quite similar to those of facilitated diffusion which suggests that a carrier mechanism is responsible for the transport process. The major difference is the occurrence of the transport against a concentration gradient. The substrate is transported from a lower chemical potential to a higher one, which is in direct opposition to the principles of diffusion. The process is thought to occur in two steps. First, an energy independent carrier mediated transport occurs, which accounts for the similarities between facilitated diffusion and active transport. This is followed by, an energy requiring accumulation step (Cirillo, 1961).

Although pinocytosis has been observed in cells growing in tissue culture, the rapid transport of small solute molecules cannot be accounted for by this process. Such a nonspecific inclusion of extracellular fluid would require hugh volumes of fluid to be engulfed to account for the amounts of solute that are taken up. Also, pinocytosis cannot account for the specificity of the transport and the kinetic observations (Christensen, 1961).

Carbohydrate transport has been studied in detail in single-celled organisms such as E. coli (Doudoroff et al., 1949, Hoffee et al., 1964, Horecker and Monod, 1960, Pardee, 1957; Osborne and McLellan, 1960), the yeast cell, S. cerevisiae, (Cirillo, 1960; Okoda and Halvorson, 1964), and the protozoan, Crithidia lucilliae (Min, 1965, 1966).

Transport in organ and tissue slices has been studied by a number

of investigators (Diedrich, 1966; Del Monto, 1961; Lassen, 1966; Morgan, 1964; Salomon et al., 1961, and Schultz et al., 1966). In most cases the results obtained in experiments with tissue and organ slices have not been consistent because of a number of difficulties, which are not within the scope of this thesis for discussion.

The red blood cell has been the object of a number of carbohydrate transport studies as representative of the mammalian cell (Lacko, et al. 1961; LeFevre, 1962; Miller, 1965; and Wilbrandt and Rosenberg, 1956).

Mammalian cells grown in tissue culture have been used to study transport in single-cell populations of mammalian cells. Maio (1962) and Maio and Rickenberg (1961, 1962) used Earle's L-strain cells derived from mouse connective tissue for extensive experiments on the galactose transport across the cell membrane.

The study of sugar transport into cancer cells has been limited to the study of the Ehrlich ascites tumor cells, a mouse carcinoma (Crane et al., 1957; Cirillo and Young, 1964; and Nirenberg and Hogg, 1958) and Vann et al (1963) have reported on glucose uptake by HeLa cells, a cell line derived from a human cervical carcinoma.

The human cancer cell was chosen for this study because, although it is a mammalian cell, it has properties that are different both morphologically and biochemically from the normal mammalian cell.

Cancer cell nuclei tend to be large relative to the cytoplasm and high in average ploidy, the nucleoli are often increased in size and number, and mitoses are increased in frequency. Irregularities are seen in the nuclear and cell membrane and the mitochondria may show loss of their internal structure (Reid, 1965).

Biochemically, cancer cells tend to dedifferentiate enzymatically. The variation for a given enzyme system between different types of tumors is smaller than between the original healthy tissues. The most important change in the biochemistry of cells that become cancerous is the adoption of a high level of anaerobic glycolysis (LeBreton and Moule, 1961). Warburg's hypothesis (1930) that the cause of the intense level of glycolysis is an injury to the respiratory system has been disputed by Agol et al (1959) and El'tsina (1960). An alternative hypothesis expressed by Reid (1965) is that the high level of glycolysis is due to the inadequate blood supply and subsequent lack of oxygen. However, Lehninger (1965) states that even with an adequate supply of oxygen the cancer cell continues to use up large quantities of glucose by glycolysis. Nonetheless, the fact remains that the production of metabolic energy by the cancer cell is principally by the glycolytic pathway.

Most studies of carbohydrate transport have utilized nonmetabolizable sugars since the chemical test for sugars will not be positive for the various derivatives produced by metabolism. In the experiments with KB cells uniformly labelled glucose was used, thus it was possible to detect glucose and all the intermediary metabolites. The KB cells are known to metabolize glucose by glycolysis (Eagle et al, 1958) thus producing as a final product 2 moles of lactic acid per mole of glucose metabolized, therefore the total intracellular radioactivity of the lactic acid and the other intermediate metabolites are equivalent to glucose. The results are expressed as the millimolar concentration of the glucose equivalents. Error due to loss of carbon as carbon dioxide is negligible in short term experiments. Crockett and Leslie (1965) have shown that

human cells grown in tissue culture lose only 10 percent of the total radioactivity as carbon dioxide in 48 hour experiments.

The kinetic analysis of the data was done with the mathematical models for transport kinetics derived by Wilbrandt and Rosenberg (1955, 1956). The kinetic types are designated as D, Z, and E (see Appendix, pages 32,33). These models are used with other criteria to determine the mechanism of carbohydrate transport in KB cells.

Type D kinetics are identical with the kinetics of simple diffusion described by Fick's Law. The model for Z kinetics assumes that the substrate reacts with a fixed membrane constituent and exhibits saturation kinetics. The model for type E kinetics is based on the formation of a complex between the substrate and a mobile membrane constituent with the participation of an enzyme, followed by the diffusion of the complex across the cell membrane. The same model can be formulated on the basis of a substrate mobile carrier reaction with subsequent diffusion of the complex across the cell membrane without the participation of an enzyme.

The data were also analyzed with the Michaelis-Menten equation (Patton, 1965) (Appendix, page 31) to demonstrate further the enzyme-like properties of the transport system and to calculate the kinetics parameters $K_M$ and $V_{Max}$.

# CHAPTER II

# MATERIALS AND METHODS

## Cells

Tissue cultured human strain KB cells derived from an oral carcinoma were obtained from the laboratory of Dr. R. H. Fetner of Georgia Institute of Technology and the cells were maintained in continuous culture on Eagle's Minimum Essential Medium (Eagle, 1959) at 35°C supplemented with nonessential amino acids, penicillin (0.5 mg/l), streptomycin (0.5 mg/l), sodium bicarbonate (0.35 g/l) and 10 percent pooled human serum from fasted patients. Hanks' salt solution (Hanks and Wallace, 1949) was substituted for Earle's. The cells were grown attached to glass in 8 ounce prescription bottles until the maximum stationary phase was reached. The growth medium was then decanted and the cells were washed three times with equal volumes of Hanks salt solution.

Packed-cell volume was determined on two different bottles in sedimentation tubes, Kimax #46815, by centrifugation for 5 minutes at 3,300 g.

## Chemicals

Uniformly labelled D-Glucose with a specific activity of 1 mc/mM was obtained from Cal Biochem. Nonradioactive reagent grade glucose was obtained from Fisher Scientific and used without further purification.

## General Procedure

To each bottle of washed cells 5 ml of twice the desired concentration of glucose and 5 ml of labelled solution (0.1 μc/ml) were added.

The bottles were incubated at 35°C and at each of the designated time intervals one bottle was removed. The cells were scraped off the glass surface with a rubber policeman. And transferred to 15 ml centrifuge tubes that had previously been coated with GE SC-87 Dri-Film and contained 0.1 ml of organic separator (Octoil and Di-n-butyl pthalate, 6:5) (Ballantine and Burford, 1960). The cells were centrifuged at 3,300 g for 1-2 minutes. The supernatant was removed and all traces of liquid were removed by swabbing with absorbent paper. The cells were then resuspended in ice cold Hanks solution and immediately centrifuged for 1 minute at 3,300 g. This step removed carbohydrate trapped in the intercellular spaces so that no correction was necessary for intercellular sugar. The supernatant was discarded and any adhering moisture was removed by swabbing. The cell pellets were resuspended in 0.8 ml of 0.1 N lithium hydroxide to disrupt the cell membrane. Then 0.1 ml each of 5 percent $ZnSO_4$ and 0.3 N $Ba(OH)_2$ was added to precipitate the proteins. The sample was centrifuged until all the precipitate had sedimented. The clear supernatant was transferred to 25 ml liquid scintillator vials, neutralized with HCl and then 15 ml of scintillation fluid were added. The intracellular water was analyzed for radioactivity with a Packard Tri Carb liquid scintillator. With each experiment a blank was made to determine background. The blank was treated as above except that no labelled solution was added. The average background was 54 cpm for the 28 experiments. A standard was prepared for each experiment by transferring 0.5 ml of twice the desired concentration of glucose and 0.5 ml of labelled solution to a scintillation vial and adding 15 ml of scintillation fluid.

# CHAPTER III

# RESULTS

## Time Course of Glucose Uptake

The KB cells were exposed to a series of four different concentrations of glucose. Samples were taken at appropriate time intervals to determine the pattern of glucose uptake by the KB cells. Figure 1 shows that the uptake is initially linear and then reaches a steady-state after 30 minutes of exposure. This steady-state is maintained by the cells for the duration of the experiment. The steady-state intracellular concentration of glucose equivalents is greater than the external concentration of glucose when the cells are incubated in 1.0 and 5.5 millimolar glucose solutions. The internal glucose equivalents at the steady-state when the cells are incubated in 10 and 20 millimolar glucose solutions is less than the external glucose concentration. Student's t test was applied to the results (Appendix, 35) to determine if the differences in external and internal steady-state concentrations are significant. The results of the test show that the steady-state concentration of intracellular glucose equivalents after an incubation time of 120 minutes is significantly different from the external concentrations of 1.0, 5.5 and 20 millimolar. A further investigation (Table 2, Appendix 34) indicates that if the cells are left in contact with a 20 mM solution for 180 minutes the internal glucose concentration approaches the external concentration. The internal steady-state concentration of cells incubated in a 10 mM glucose solution

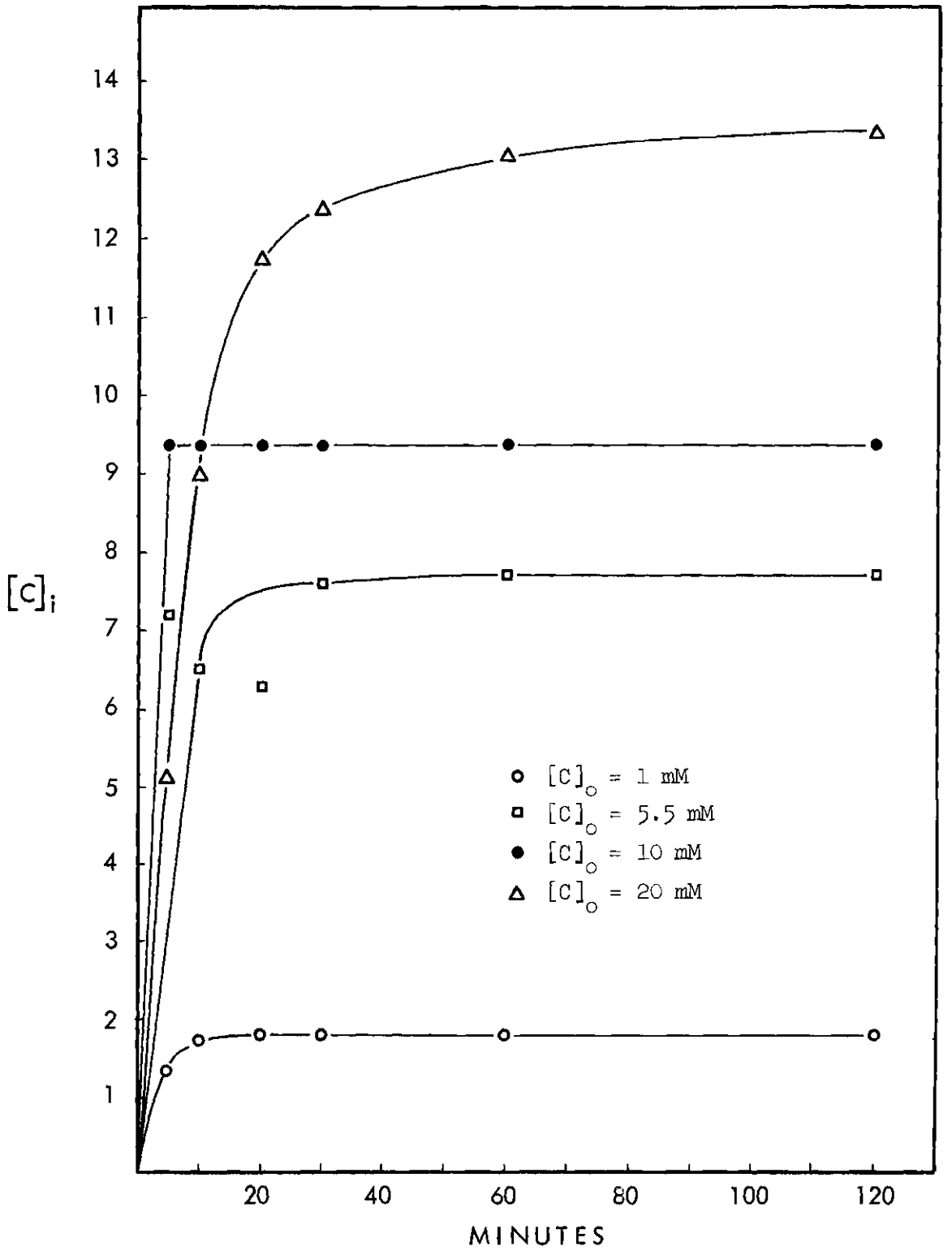



Figure 1. Relationship between Intracellular Concentration and Time of Exposure.

is not significantly different from the external concentration.

## Saturation Kinetics

Inspection of Figure 1 reveals that an increase in the external concentration does not result in a proportionate increase in uptake by cells. To further elucidate the effect of external glucose concentration on uptake the initial rate of uptake (mM glucose equivalents/ml. PCV/minute) was calculated at each external concentration studied. Figure 2 shows the effect of the external concentration on rate of transport of glucose. At concentrations lower than 10 mM the rate of uptake increases nearly linearly with increasing concentration. However, increasing the external concentration beyond 10 mM does not result in an increase in the velocity.

## Distribution of Glucose between Cells and Suspending Medium

The distribution ratio $[G]_i/[G]_o$ was calculated at the steady-state to determine the effect of external glucose concentration on the distribution of glucose between cells and medium. Figure 3 shows that the distribution ratio decreases when the concentration of glucose in the external solution is increased. The distribution ratios at 1 and 5.5 millimolar are 1.8 and 1.4 respectively, indicating that the cells are accumulating glucose from solutions of low concentrations.

## Kinetic Analysis

The data obtained in these experiments were analyzed by the kinetic models for transport derived by Rosenberg and Wilbrandt (1955). The curves predicted by the models for types D, Z, and E kinetics of transport are compared with the actual uptake curves. The probability that the observed

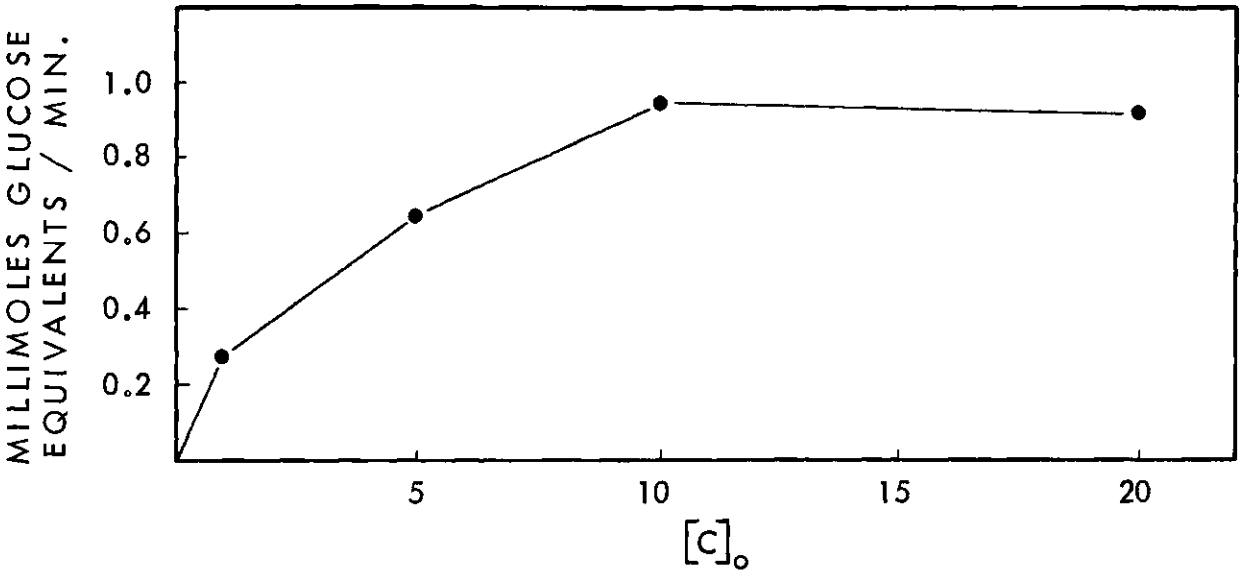



Figure 2. Effect of External Glucose Concentration on the Velocity of Transport.

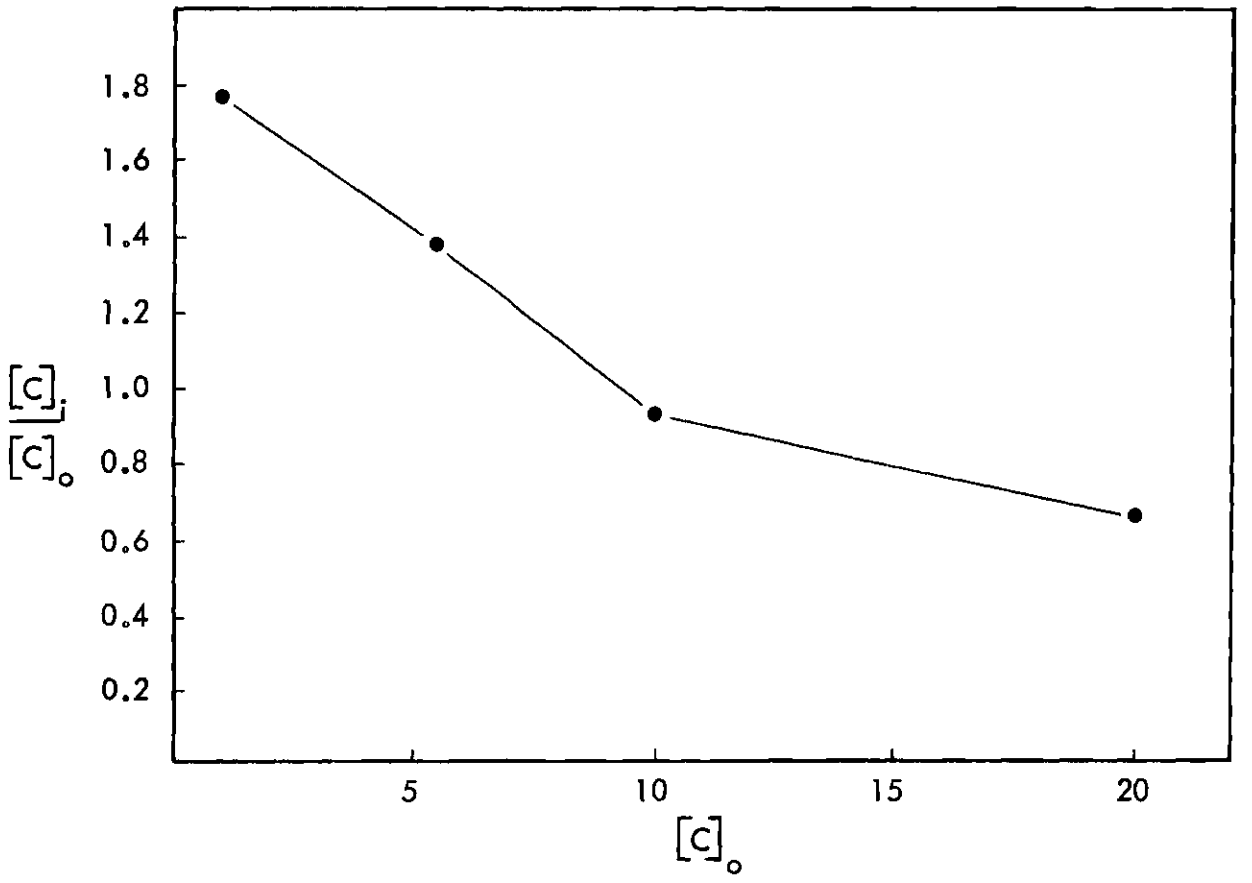



Figure 3. The Effect of the External Glucose Concentration on the Distribution Ratio.

kinetics are the same as the kinetics predicted by the model is determined from a Chi Square analysis. The determination of the kinetics of glucose transport into KB cells will allow some conclusions to be made as to the mechanism of the transport on the basis of present transport theory.

D Kinetics

Figure 4 compares the kinetics of uptake from glucose solutions of 1, 5.5, 10, and 20 millimolar solutions with predicated kinetic pattern for type D (Fick's Law diffusion) transport. Uptake from a 20 mM glucose solution follows the kinetic pattern of type D transport ($P = 0.78$), uptake from a 10 mM solution does not conform to the type D pattern ($P = 0.04$) and it appears that uptake from 5.5 and 1.0 millimolar solutions follow some other type of kinetics.

$Z_1$ Kinetics

The kinetics of uptake from 10 and 20 millimolar solutions are compared with the type $Z_1$ kinetics in Figure 5. The observed kinetics are not of the $Z_1$ type ($P < 0.01$). The kinetics of uptake from 1.0 mM and 5.5 mM solutions were not studied because the model does not hold for internal concentrations greater than the external concentration of substrate.

E Kinetics

Figure 6 compares the kinetics of the KB cell transport system with the type E kinetics. Uptake from the 10 mM solution is of the E kinetic type ($P = 0.99$); however, the uptake from the 20 mM solution is not of the E type ($P < 0.01$).

Michaelis-Menten Kinetics

Figure 6 indicates that uptake from a 10 mM solution of glucose

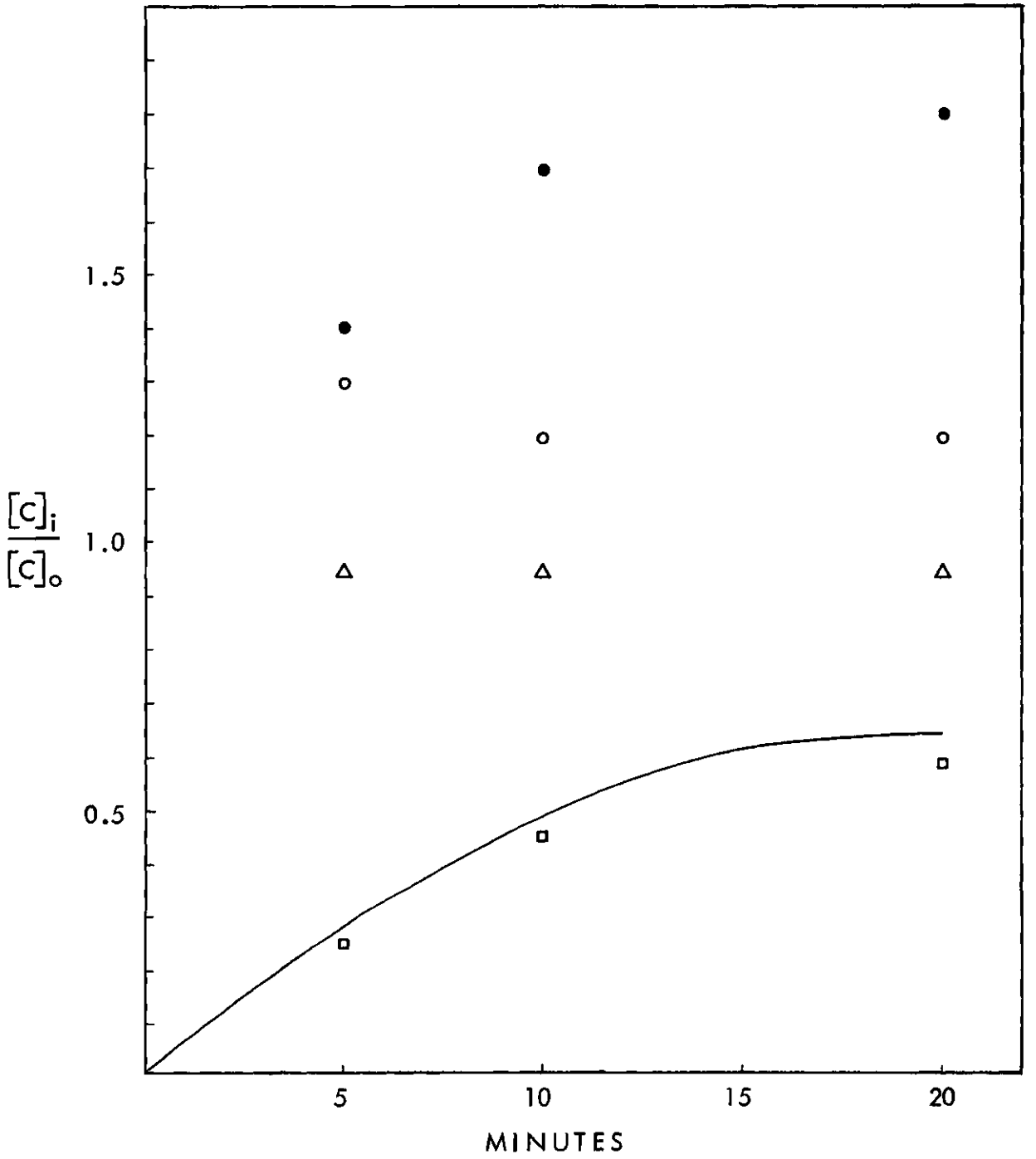



Figure 4. Comparison of D and Observed Kinetics.

- ● $[C]_o$ = 1.0 mM
- ○ $[C]_o$ = 5.5 mM
- △ $[C]_o$ = 10 mM
- □ $[C]_o$ = 20 mM
- —— theoretical D kinetics

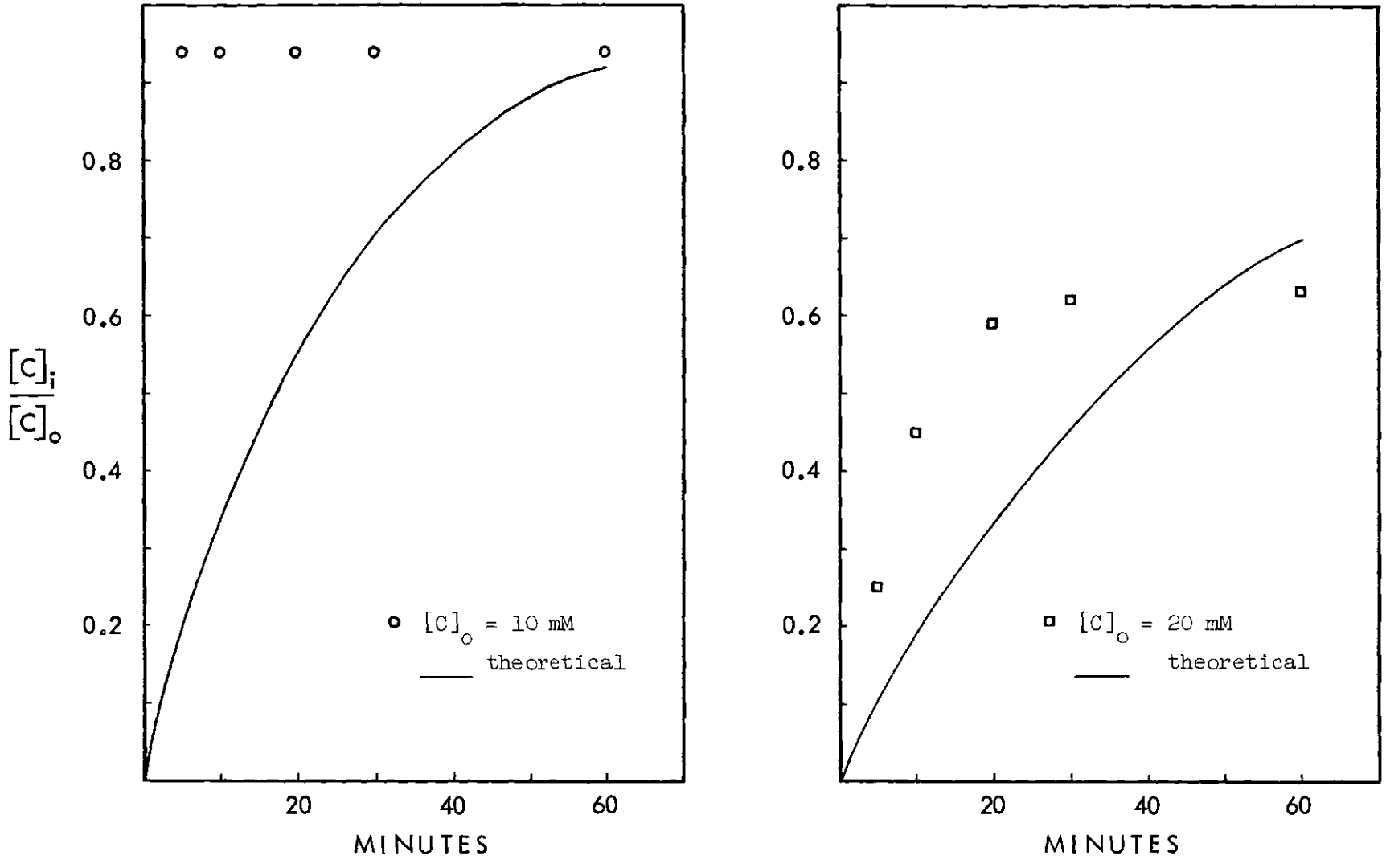



Figure 5. Comparison of $Z_1$ and Observed Kinetics.

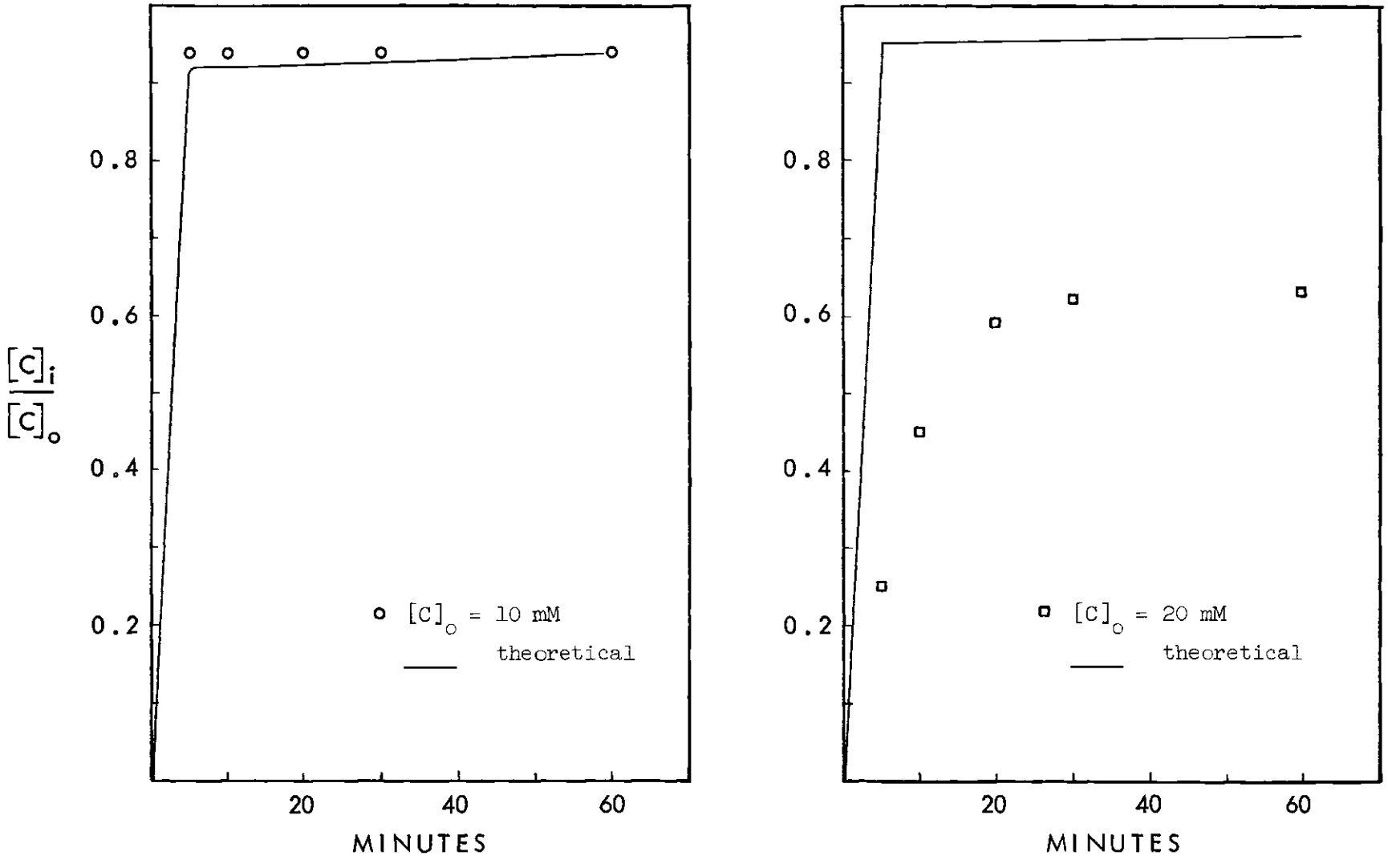



Figure 6. Comparison of E and Observed Kinetics.

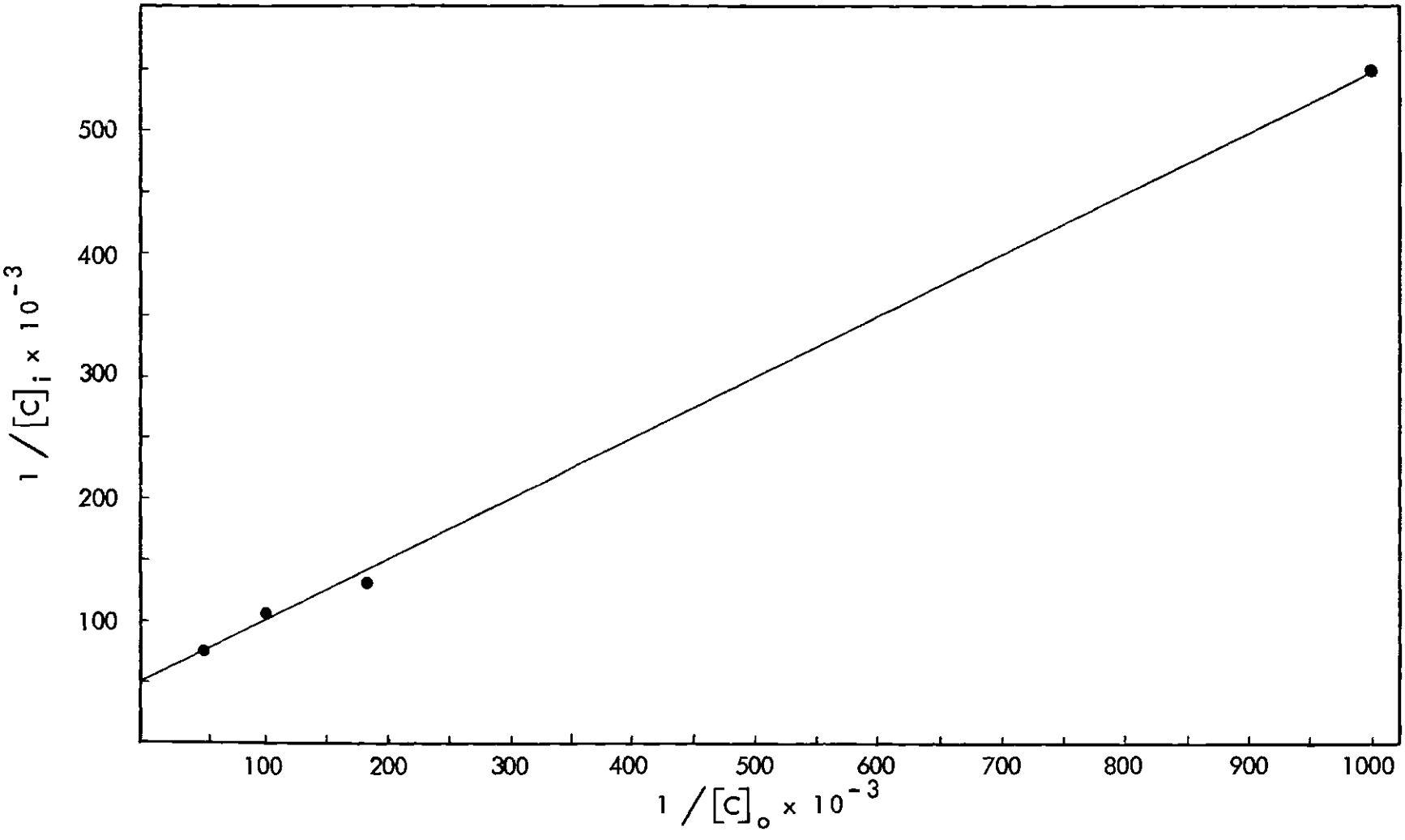



Figure 7. Effect of External Glucose Concentration on Glucose Uptake.

followed enzyme-like kinetics. It is suspected that the transport at all external concentrations follows enzyme-like kinetics. If the transport system does follow enzyme kinetics over all ranges of external concentration the data will fit the Michaelis-Menten equation of enzyme kinetics. In Figure 7 external glucose concentration is plotted against the concentration of intracellular glucose equivalents in the reciprocal form of the Michaelis-Menten equation derived by Lineweaver and Burk (Patton, 1965). A least mean square line was calculated from the means of the experimental uptake data and excellent agreement to Michaelis-Menten kinetics is shown. From the intercept and slope of the least mean square line the kinetic parameters; Michaelis constant ($K_M$ = 10 mM) and the maximum velocity ($V_{Max}$ = 20 mM/min) were calculated. The Michaelis constant represents the affinity of the transport system for the substrate and $V_{Max}$ is the theoretical maximum attainable velocity of transport.

Adsorption Isotherm

Figure 8 indicates that the kinetics of glucose transport into KB cells follows an adsorbtion isotherm (Langmuir, 1918).

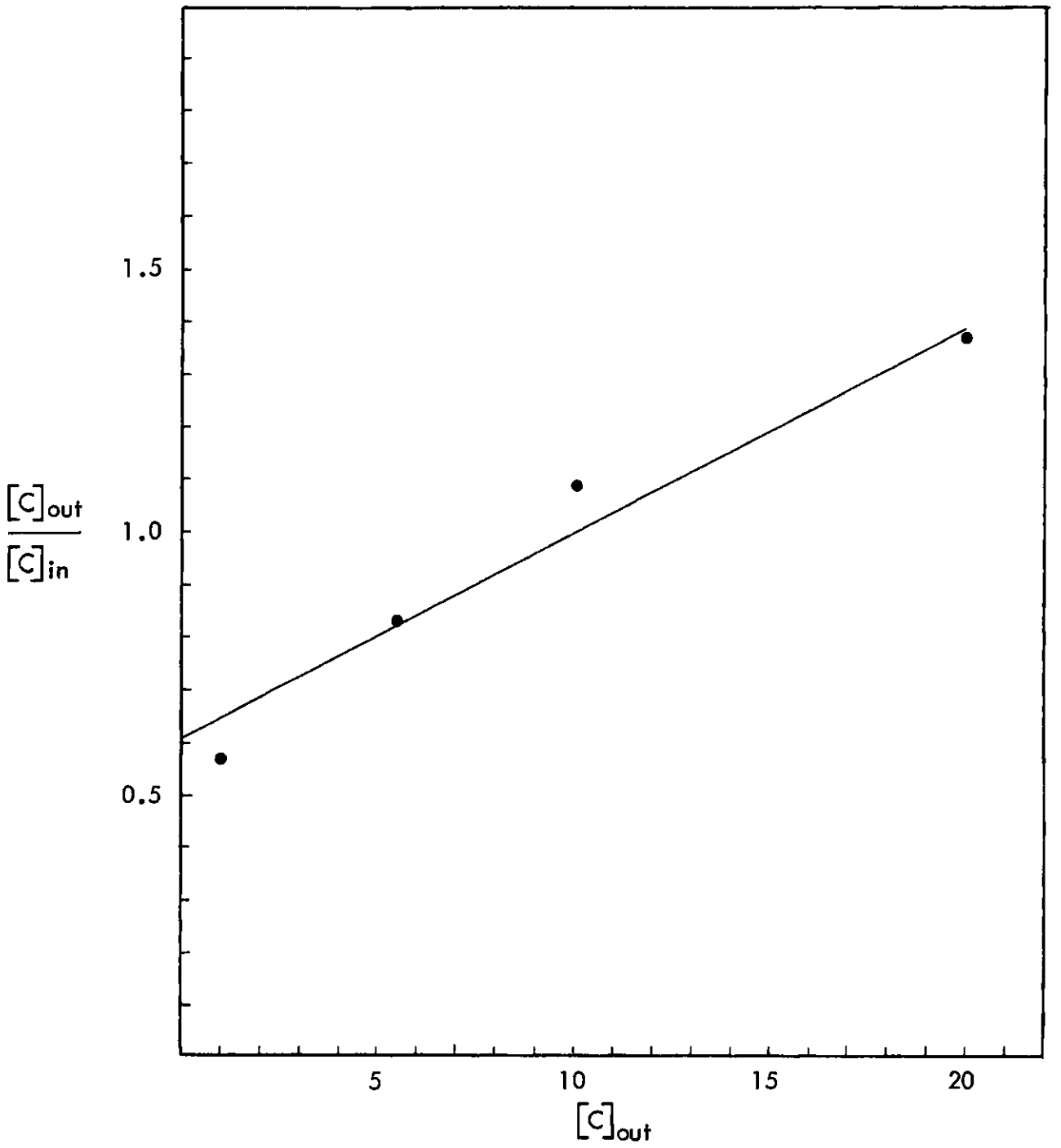



Figure 8. Langmuir Adsorption Isotherm.

# CHAPTER IV

## DISCUSSION

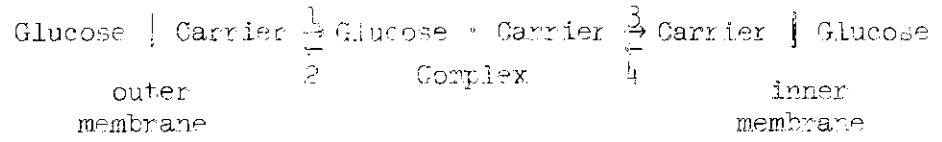
The transport of glucose into KB cells exhibit the following characteristics:

1) Uptake follows Michaelis-Menten kinetics.
2) Uptake does not increase linearly with the increase in external glucose concentration and saturation kinetics are observed.
3) The kinetics of glucose transport follow an adsorption isotherm.
4) Uptake from a 10.0 mM glucose solution follows type E kinetics, while uptake from a 20.0 mM solution follows type D kinetics.
5) The KB cell apparently possesses the ability to concentrate glucose from dilute solutions.

The above characteristics of the glucose transport system in the KB cells are not in accord with the criteria for simple diffusion. The observed pattern of glucose uptake is similar to the carrier mediated transport observed in other cell types by Cirillo (1961), Maio (1962) and Min (1965).

Based on the observations in these experiments the following model proposed by Cirillo (1961) may represent the mechanism of glucose transport in KB cells. This model, although the simplest of all the proposed

mechanisms of mediated transport, can account for all the kinetic observations made in these experiments.




The kinetics of glucose transport follow an adsorption isotherm indicating that the glucose molecule is reversibly adsorbed onto the surface of a membrane component before diffusing across the cell membrane. A molecular adsorption process may, therefore, be the nature of the proposed carrier-substrate complex formation. The mechanism would also obey enzyme kinetics.

Saturation kinetics are observed in glucose transport when the external concentration of glucose is increased. This result indicates that the membrane component which complexes with the glucose molecule is present in only limited amounts.

The kinetics of the uptake pattern of glucose by the KB cells follows initially first order kinetics changing to zero order behavior at longer incubation times. This same type of pattern is observed with enzymatic reactions. Glucose transport into KB cells also follows Michaelis-Menten kinetics. The kinetic patterns observed in glucose uptake by the KB cells indicate that an enzymatic type reaction occurs in the transport process.

Further analysis of the data with mathematical models for transport kinetics show that uptake from a 10.0 mM solution follows E-type kinetics which are based on the assumptions of an enzymatic step and the participation of a mobile carrier. Uptake from a 20.0 mM solution follows D-type

kinetics which is based on the criteria for simple diffusion and is identical with Fick's Law. This does not mean that the carrier mechanism ceases to function at high substrate concentration.

At low glucose concentrations the equilibrium reactions 1 and 2 as well as 3 and 4 (see model) are important and the rate-limiting step is the carrier-glucose complex formation involving reactions 1 and 2, the reverse reaction (2) is significant. When the substrate concentration is very high ($[C]_o > K_M$), reaction (2), becomes supressed. The reaction is accelerated in the direction of reaction (1) and thus the complex reaction is not rate-limiting. In this case the diffusion of the complex across the membrane becomes the rate-limiting step for the transport.

KB cells apparently possess the ability to concentrate glucose from dilute solutions. This ability has also been observed in protozoan cells (Min, 1965), in yeast cells (Okada, 1964), and in mammalian cells grown in tissue culture (Rickenberg and Maio, 1961). It was not within the scope of this work to determine if the transport is dependent on metabolic energy or if the glucose is osmotically active in the cell. Therefore, it would be premature at this point to say that an active transport mechanism is operating.

Table 1 is a comparison of the affinity constants, $K_M$, for glucose transport in several different types of cells. All of the cell types presented in this table metabolize glucose by glycolysis.

The carbohydrate transport system of the human cancer cell, KB, does not exhibit any unusual properties in comparison with other mammalian cell types. The transport systems of Ehrlich ascites and the Earle's

Table 1. Michaelis-Menten Constants for Glucose Transport

| Cell Type | $K_M$ (mM) | Reference |
|---|---|---|
| Mammalian tumor | | |
| KB | 10.0 | this paper |
| Ehrlich ascites | 0.7 | Crane |
| Mammalian tissue culture | | |
| L-strain | 1.0 | Maio |
| Muscle | | |
| Frog sartorius | 6.0 | Narahara* |
| Rat heart | 6.6 | Morgan* |
| Erythrocyte | | |
| Human | 7.0 | LeFevre* |
| Human | 10.0 | Widdas* |
| Human | 5.0 | Reinwein* |
| Human | 4.0 | Sen & Widdas* |
| Human | 7.0 | Wilbrandt* |
| Rabbit | 4.0 | Park |

*Data obtained from Henderson, 1964.

L-strain cells show slightly greater affinities for glucose. The other cell types listed in Table 1 show no significantly different affinities. The L, KB, and Ehrlich strains are all anaerobes, whereas, erythrocytes and muscle cells are aerobes, therefore the differences in affinity for glucose cannot be attributed to metabolic differences.

Although the biochemistry and physiology of the human cancer cell are definitely different from normal body cells, the carbohydrate transport system is apparently not disturbed.

Because of high mitotic activity, cancer cells would be expected to have greater energy requirements than normal cells. Quantitatively, energy production per mole of glucose is only one-nineteenth of the energy produced per mole in respiration. Therefore, to supply the cancer cell with the necessary energy, an increase in the activity of the glucose transport system might be expected. However, the results obtained in these experiments seem to support Eagle's statement (1955) that in isolated cells transport across the cell membrane is not rate limiting for glycolysis and that, therefore, sugar entry is not a site of regulatory control of cellular metabolism.

# CHAPTER V

## CONCLUSIONS

1) The transport system in KB cells does not appear to be anomalous.

2) The kinetic analysis of the data indicates that the transport mechanism possesses enzyme-like properties but this does not necessarily mean that an enzyme is actually involved.

3) The transport kinetics follow an adsorption isotherm, which indicates that the glucose is adsorbed to a membrane constituent, this process would also follow enzyme kinetics.

4) Transport in KB cells appears to be mobile carrier mediated, similar to the systems observed in other cell types.

5) There appears to be no functional relationship between the biochemical and physiological state of the KB line cancer cell and its carbohydrate transport system.

# CHAPTER VI

## RECOMMENDATIONS

The effect of metabolic inhibitors, temperature, and structural analogues on the transport of glucose into the KB cells should be studied to ellucidate more fully the mechanism of the transport system and to substantiate the results obtained from the kinetic analysis.

The existence of a counterflow mechanism should be shown to prove conclusively that the membrane carriers are mobile.

# APPENDIX

## EAGLES MINIMUM ESSENTIAL MEDIA

Essential Amino Acids

| | |
|---|---|
| L-Arginine | 105 mg/l |
| L-Histidine | 31 mg/l |
| L-Isoleucine | 52 mg/l |
| L-Leucine | 52 mg/l |
| L-Lysine | 58 mg/l |
| L-Methionine | 15 mg/l |
| L-Phenylalamine | 32 mg/l |
| L-Threonine | 48 mg/l |
| L-Tryptophan | 10 mg/l |
| L-Valine | 46 mg/l |
| L-Tyrosine | 36 mg/l |
| L-Cystine | 24 mg/l |

Nonessential Amino Acids

| | |
|---|---|
| L-Alamine | 8.9 mg/l |
| L-Aspargine | 13.2 mg/l |
| L-Aspartic acid | 13.3 mg/l |
| L-Glutonic acid | 14.7 mg/l |
| L-Proline | 11.5 mg/l |
| L-Serine | 10.5 mg/l |

These amino acids are made up in a 20 x solution and frozen in 50 ml aliquots.

Vitamins

| | |
|---|---|
| Thiamine | 1.0 mg/l |
| Riboflavin | 0.1 mg/l |
| Choline | 1.0 mg/l |
| Pyridoxal | 1.0 mg/l |
| Folic Acid | 1.0 mg/l |
| Inosital | 2.0 mg/l |
| Nicotinamide | 1.0 mg/l |
| Pantothenate | 1.0 mg/l |

Vitamins made up 200 x frozen in liquid nitrogen in 5 ml vials.

Antibiotics

| | |
|---|---|
| Penicillin | 0.5 mg/l |
| Streptomycin | 0.5 mg/l |

Other Ingredients

| | | |
|---|---|---|
| Glucose | 1 g/l | |
| Phenol Red | .0.02 g/l | |
| (Make up 20 x soln. of) | | |
| $NaHCO_3$ | .0.35 g/l | |
| L-Glutamine | .292 mg/l | - added when media is made up. |
| Human Serum | .100 ml/l | |

Phosphate Buffer

| | |
|---|---|
| $Na_2HPO_4$ | .0.006 g/l |
| $KH_2PO_4$ | .0.06 g/l |

Make up 20 x solution.

Hanks Balance Salt Solution

| Salts | g/l |
|---|---|
| NaCl | 8 |
| KCl | 0.4 |
| $CaCl_2$ | 0.14 |
| $MgSO_4$ | 0.10 |
| $MgCl_2$ | 0.10 |

(Make 10 X solution)

## LIST OF SYMBOLS

| | |
|---|---|
| $[C]_i$ | intracellular concentration of glucose equivalents - millimoles/milliliter PCV |
| $[C]_o$ | extracellular concentration of glucose-millimoles |
| PCV | packed-cell volume-milliliters |
| $V_{Max}$ | theoretical maximum attainable velocity of transport millimoles/minute |
| $K_D$ | Fick's Law Diffusion constant - liters/second |
| $K_Z$ | constant for Z kinetics - (millimoles)/minute |
| $K_E$ | constant for E kinetics - $(\text{millimoles})^2$/minute |
| $K_M$ | Michaelis-Menten constant - millimoles |
| CPM | counts per minute |
| t | minutes |

# LIST OF EQUATIONS

Calculation of intracellular concentration

$$[C]_i = [C]_o \times \frac{\text{net CPM of sample}}{\frac{\text{CPM of 1 ml of media}}{\text{PCV}}}$$

Kinetic Equations

D $\quad [C]_i/[C]_o = 1 - e^{-K_D t}$

$Z_1$ $\quad [C]_i/[C]_o = 1 - e^{-K_Z t/[C]_o}$

E $\quad [C]_i/[C]_o = 1 - \frac{[C]_o}{[C]_o^2 \cdot K_E t}$

Michaelis-Menten (Lineweaver-Burk reciprocal form)

$$1/[C]_i = K_M/V_{Max} \; (1/[C]_o) + 1/V_{Max}$$

Langmuir's Adsorption Isotherm

$$[C]_o/[C]_i = 1/ab + [C]_o/b$$

# DERIVATION OF KINETIC EQUATIONS

## D-Kinetics or Ficks Law

$$v = K_D([C]_o - [C]_i)$$

$$v = d[C]_i/dt$$

$$d[C]_i/([C]_o - [C]_i) = K_D dt$$

$$\int_o^{[C]_i} d[C]_i/([C]_o - [C]_i) = K_D \int_o^{[C]_i} dt$$

$$\ln [C]_o/([C]_o - [C]_i) = K_D t$$

$$([C]_o - [C]_i) = e^{-K_D t}$$

$$[C]_i/[C]_o = 1 - e^{-K_D t}$$

## $Z_1$ - Kinetics

$$v = K_Z([C]_o - [C]_i)$$

$$v = d[C]_i/dt$$

$$d[C]_i/dt = K_Z ([C]_o - [C]_i)/[C]_o$$

$$[C]_o \int_o^{[C]_i} d[C]_i/([C]_o - [C]_i) = K_Z \int_o^t dt$$

$$[C]_o(\ln[C]_o/([C]_o - [C]_i)) = K_Z t$$

$$\ln[C]_o/([C]_o - [C]_i) = K_Z t/[C]_o$$

$$[C]_i/[C]_o = 1 - e^{-K_Z t/[C]_o}$$

E-Kinetics

$$v = K_E\,(1/[C]_i - 1/[C]_o)$$

$$d[C]_i/dt = K_E\,([C]_o - [C]_i)/[C]_i[C]_o$$

$$[C]_o\int_o^{[C]_i}[C]_i\,d[C]_i/([C]_o - [C]_i) = K_E\int_o^t dt$$

$$-[C]_o\,([C]_o - [C]_i - [C]_o \ln([C]_o - [C]_i)) = K_E t$$

$$[C]_i + [C]_o \ln([C]_o - [C]_i) = K_E t/[C]_o + [C]_o$$

$$[C]_i/[C]_o + \ln([C]_o - [C]_i) = (K_E t + [C]_o^2)/[C]_o^2$$

log series approximation

$$[C]_i/[C]_o + ([C]_o - [C]_i - 1)/([C]_o - [C]_i) = (K_E t + [C]_o^2)/[C]_o^2$$

rearranging

$$[C]_i/[C]_o = 1 - [C]_o/([C]_o^2 - K_E t)$$

Table 2. The Time Course of Glucose Uptake

| Extracellular $[C]_o$ | Time Min. | Mean $[C]_i$ | No. of Experiments | Standard Error ± |
|---|---|---|---|---|
| 1.0 mM | 5 | 1.4 | | 0.03 |
| | 10 | 1.7 | 5 | 0.04 |
| | 20 | 1.8 | | 0.04 |
| | 30 | 1.8 | | 0.04 |
| | 60 | 1.8 | | 0.04 |
| | 120 | 1.8 | | 0.22 |
| 5.5 mM | 5 | 7.20 | | 1.28 |
| | 10 | 6.46 | | 0.89 |
| | 20 | 6.28 | 5 | 0.76 |
| | 30 | 7.62 | | 1.31 |
| | 60 | 7.70 | | 1.26 |
| | 120 | 7.66 | | 1.49 |
| 10.0 mM | 5 | 9.4 | | 0.03 |
| | 10 | 9.4 | | 0.08 |
| | 20 | 9.4 | 5 | 0.17 |
| | 30 | 9.4 | | 0.37 |
| | 60 | 9.4 | | 0.14 |
| | 120 | 9.4 | | 0.88 |
| 20 mM | 5 | 5.09 | | 0.55 |
| | 10 | 9.01 | | 1.10 |
| | 20 | 11.77 | 7 | 0.91 |
| | 30 | 12.37 | | 0.83 |
| | 60 | 13.11 | | 1.15 |
| | 120 | 13.40 | | 1.15 |
| | 180 | 14.94 | | 0.97 |

STUDENT'S t TEST

| $[c]_i$ | $[c]_o$ | $\Delta$ | $\Delta^2$ |
|---|---|---|---|
| 14 | 20 | 6 | 36 |
| 14 | 20 | 6 | 36 |
| 11.4 | 20 | 8.6 | 74 |
| 11.9 | 20 | 8.1 | 64 |
| 15.5 | 20 | 4.5 | 20 |
| 18.2 | 20 | 1.8 | 3 |
| 8.8 | 20 | 11.2 | 121 |
| 93.8 | 140 | | 354 |

$\bar{[c]}_i = 13.4 \quad \bar{[c]}_o = 20 \quad SE_\Delta = \frac{\sqrt{\frac{\Sigma\Delta^2}{N-1}}}{\sqrt{N}} = \frac{\sqrt{\frac{357}{6}}}{\sqrt{7}} = 2.98$

$$t = \frac{\bar{[c]}_o - \bar{[c]}_i}{SE_\Delta} = \frac{6.6}{2.98} = 2.2$$

$10\% > P > 5\%$

| $[c]_i$ | $[c]_o$ | | |
|---|---|---|---|
| 15.5 | 20 | 4.5 | 20 |
| 11.9 | 20 | 8.1 | 64 |
| 14.4 | 20 | 5.6 | 36 |
| 13.0 | 20 | 7.0 | 49 |
| 15.3 | 20 | 4.7 | 22 |
| 14.5 | 20 | 5.5 | 30 |
| 20.0 | 20 | 0.0 | 0 |
| 104.6 | 140 | | 221 |

$\bar{[c]}_i = 14.9 \quad \bar{[c]}_o = 20.0 \quad SE_\Delta = \frac{\sqrt{\frac{\Sigma\Delta^2}{N-1}}}{\sqrt{N}} = \frac{37}{2.6} = 14$

$$t = \frac{[\bar{C}]_o - [\bar{C}]_i}{SE} = \frac{5.1}{14} = 0.36$$

$80\% > P > 70\%$

| $[C]_i$ | $[C]_o$ | | $\Delta$ | $\Delta^2$ |
|---|---|---|---|---|
| 9.0 | 10 | | 1.0 | 1.0 |
| 12.8 | 10 | 2.8 | 2.8 | 7.8 |
| 7.9 | 10 | | 2.1 | 4.0 |
| 8.4 | 10 | | 1.6 | 2.6 |
| 38.1 | 40 | | | 15.4 |

$$[\bar{C}]_i = 9.4 \quad [\bar{C}]_o = 10.0 \quad SE_\Delta = \frac{\sqrt{\frac{\Sigma\Delta^2}{N-1}}}{\sqrt{N}} = \frac{2.2}{2} = 1.1$$

$$t = \frac{[\bar{C}]_o - [\bar{C}]_i}{SE_\Delta} = \frac{0.6}{1.1} = 0.54$$

$P \approx 70\%$

| $[C]_i$ | $[C]_o$ | $\Delta$ | $\Delta^2$ |
|---|---|---|---|
| 1.6 | 1.0 | 0.6 | 0.36 |
| 1.9 | 1.0 | 0.9 | 0.81 |
| 2.4 | 1.0 | 1.4 | 1.96 |
| 1.4 | 1.0 | 0.4 | 0.16 |
| 7.3 | 4.0 | | 3.29 |

$$[\bar{C}]_i = 1.8 \quad [\bar{C}]_o = 1.0 \quad SE_\Delta = \frac{\sqrt{\frac{\Sigma\Delta^2}{N-1}}}{\sqrt{N}} = \frac{1.14}{2} = .57$$

$$t = \frac{[\bar{C}]_i - [\bar{C}]_o}{SE_\Delta} = \frac{0.8}{0.57} = 1.41$$

$30\% > P > 20\%$

| $[C]_i$ | $[C]_o$ | $\Delta$ | $\Delta^2$ |
|---|---|---|---|
| 4.8 | 5.5 | 0.7 | 0.49 |
| 6.1 | 5.5 | 0.6 | 0.36 |
| 4.9 | 5.5 | 0.6 | 0.36 |
| 10.9 | 5.5 | 5.4 | 29.16 |
| 11.6 | 5.5 | 6.1 | 36.00 |
| 38.3 | 27.5 | | 66.37 |

$$\bar{[C]}_i = 7.66 \quad \bar{[C]}_o = 5.5 \quad SE_\Delta = \frac{\sqrt{\frac{\Sigma\Delta^2}{N-1}}}{\sqrt{N}} = \frac{4.07}{2.2} = 1.85$$

$$t = \frac{\bar{[C]}_i - \bar{[C]}_o}{SE_\Delta} = \frac{2.11}{1.85} = 1.2$$

$P \approx 30\%$

Table 3. Chi Square Analysis for Type D Kinetics Curve

| Extracellular Concentration | Time | $C_{in}^{Calc}$ | $C_{in}^{obs}$ | d | $d^2$ | $d^2/C_{in}^{Calc}$ |
|---|---|---|---|---|---|---|
| 20.0 mM | 5 | 5.5 | 5.1 | 0.5 | 0.25 | 0.04 |
| | 10 | 9.6 | 9.0 | 0.6 | 0.36 | 0.03 |
| | 20 | 14.6 | 11.8 | 2.8 | 7.84 | 0.53 |
| $\chi^2$ = 0.50 df = 3-1 = 2 | | | | | | |
| P = 0.780 | | | | | | |
| 10.0 mM | 5 | 2.8 | 9.4 | -6.6 | 33.56 | 3.56 |
| | 10 | 4.8 | 9.4 | -4.6 | 21.16 | 2.24 |
| | 20 | 7.3 | 9.4 | -2.1 | 4.41 | 0.48 |
| $\chi^2$ = 6.28 df = 3-1 = 2 | | | | | | |
| P = 0.045 | | | | | | |

Table 4. Chi Square Analysis for $Z_1$ Kinetics

| Time | Theor. | OBS | Theo.-obs | $(Theo.-Obs)^2$ | $(Theo.-Obs)^2/Theor.$ |
|---|---|---|---|---|---|
| 5 | 2.0 | 9.4 | 7.4 | 54.76 | 27.38 |
| 10 | 3.3 | 9.4 | 6.1 | 37.21 | 11.27 |
| 20 | 5.5 | 9.4 | 3.9 | 15.21 | 3.90 |
| 30 | 7.0 | 9.4 | 2.4 | 5.76 | 2.40 |
| 60 | 9.1 | 9.4 | 0.3 | 0.09 | 0.01 |

$X^2 = 44.56$

$P < 0.01$

| Time | Theor. | OBS | Theo.-obs | $(Theo.-Obs)^2$ | $(Theo.-Obs)^2/Theor.$ |
|---|---|---|---|---|---|
| 5 | 2.0 | 5.1 | 3.1 | 9.61 | 4.81 |
| 10 | 3.8 | 9.0 | 5.2 | 27.04 | 7.06 |
| 20 | 6.6 | 11.8 | 5.2 | 27.04 | 4.09 |
| 30 | 9.0 | 12.4 | 3.4 | 11.56 | .93 |
| 60 | 14.0 | 12.6 | 1.4 | 1.96 | .15 |

$X^2 = 17.04$

$P < 0.01$

Table 5. Chi Square Analysis for Type E Kinetics

| Time | Theor. | OBS | Theor.-Obs. | $(Theor.-Obs)^2$ | $(Theor.-Obs)^2/Theor.$ |
|---|---|---|---|---|---|
| 5 | 9.1 | 9.4 | 0.3 | 0.9 | .009 |
| 10 | 9.2 | 9.4 | 0.2 | .04 | .004 |
| 20 | 9.2 | 9.4 | 0.2 | .04 | .004 |
| 30 | 9.2 | 9.4 | 0.2 | .04 | .004 |
| 60 | 9.4 | 9.4 | 0.0 | 0.00 | 0.00 |

$\chi^2 = .021$

$P < 0.99$

20 mM

| Time | Theor. | OBS | Theor.-Obs. | $(Theor.-Obs)^2$ | $(Theor.-Obs)^2/Theor.$ |
|---|---|---|---|---|---|
| 5 | 19.0 | 5.1 | 13.9 | 193.2 | 10.2 |
| 10 | 19.0 | 9.0 | 10.0 | 100.0 | 5.3 |
| 20 | 19.0 | 11.8 | 7.2 | 51.8 | 2.7 |
| 30 | 19.0 | 12.4 | 6.6 | 43.6 | 2.3 |
| 60 | 19.2 | 12.6 | 6.6 | 43.6 | 2.3 |

$\chi^2 = 22.8$

$P < 0.01$

Table 6. Least Mean Square Line for Lineweaver-Burk plot Figure.

| $1/C_{out}$ | $1/C_{in}$ | $1/C_{in} \times C_{out}$ | $(1/C_{out})$ |
|---|---|---|---|
| 1.000 | .550 | .550 | 1.00 |
| 0.181 | .130 | .024 | 0.033 |
| 0.100 | .110 | .011 | 0.010 |
| 0.050 | .075 | .004 | 0.003 |

$\Sigma x = 1.331$ $\Sigma Y = .865$ $\Sigma XY = .589$

$\bar{X} = .333$ $\bar{Y} = .216$ $\Sigma X^2 = 1.043$

$$b = \frac{.589 - .33\ (.865)}{1.043 - .33\ (1.33)} = \frac{.304}{604} = .503$$

$Y = .216 + .503\ x - .166$

$Y = .050 + .503\ x$

Table 7. Least Mean Square Line for Adsorbtion Isotherm

| $C_{out}$ | $C_{out}/C_{in}$ | $C_{out} \times C_{out}/C_{in}$ | $C_{out}^2$ |
|---|---|---|---|
| 1.00 | 0.570 | .570 | 1.00 |
| 5.5 | 0.829 | 4.559 | 30.25 |
| 10.0 | 1.087 | 10.870 | 100.00 |
| 20.0 | 1.370 | 27.400 | 400.00 |

$\Sigma x = 36.5$ $\Sigma Y = 3.856$ $\Sigma XY = 43.399$

$\bar{x} = 9.13$ $\bar{Y} = .964$ $X^2 = 531$

$$b = \frac{43.4 - 9.1\ (3.9)}{531 - 9.1\ (36.5)} = \frac{7.9}{199} = .039$$

$Y = .964 + .039\ x - .039\ (9.13)$

$= .608 + .039\ x$

```
BEGIN
      FILE IN CRDO2(2,10) ;
      FILE OUT PTRO2 6(2,15) ;
      INTEGER I,J,COUT,N,M,P   ;
      REAL SUM,SUMDIFFSQ         ;
FORMAT
 FIN1(I5),FIN2(I5),FIN3(10F6.2),FIN4(I5),FIN5(10F6.2),FIN6(F4.1) ,
 FOUT1("COUT",F4.1,"MILLIMOLAR"),
 FOUT2(//,X10,"TIME",X6,"MEANCONC",X5,"VAR",X8,"STDDEV",X6,"STDERROR"),
 FOUT3(X9,I4,6(X8F5.2))  ;
      LABEL L1,L2    ;
      REAL ARRAY CIN[0:20,0:20] ;
      LIST LST1(FOR I:=1 STEP 1 UNTIL N DO
                FOR J:=1 STEP 1 UNTIL M DO CIN[I,J]) ;
      REAL ARRAY T[0:20]  ;
      LIST LST2(FOR I:=1 STEP 1 UNTIL P DO T[I]) ;
      REAL ARRAY SUMROW[0:20] ;
      REAL ARRAY MEAN[0:20] ;
      REAL ARRAY VAR[0:20] ;
      REAL ARRAY SD[0:20] ;
      REAL ARRAY SE[0:20] ;
      LIST LST3(T[I],MEAN[I],VAR[I],SD[I],SE[I]) ;
      WRITE(PTRO2[NO1) ;
   L2:READ(CRDO2,FIN1,N) (L1) ;
      READ(CRDO2,FIN2,M) ;
      READ(CRDO2,FIN3,LST1) ;
      READ(CRDO2,FIN4,P) ;
      READ(CRDO2,FIN5,LST2) ;
      READ(CRDO2,FIN6,COUT) ;

      FOR I:=1 STEP 1 UNTIL N DO
        BEGIN
             SUM:=0 ;
             FOR J:=1 STEP 1 UNTIL M DO SUM:=SUM+CIN[I,J] ;
             SUMROW[I]:=SUM
        END ;
      MEAN[I]:=0 ;
      FOR I:=1 STEP 1 UNTIL N DO MEAN[I]:=SUMROW[I]/M ;
      FOR I:=1 STEP 1 UNTIL N DO
        BEGIN
            SUMDIFFSQ:=0 ;
            FOR J:=1 STEP 1 UNTIL M DO
             SUMDIFFSQ:=SUMDIFFSQ+(CIN[I,J]-MEAN[I])**2 ;
             VAR[I]:=SUMDIFFSQ/(M-1)
        END ;
      FOR I:=1 STEP 1 UNTIL N DO
         SD[I]:=SQRT(VAR[I]) ;
      FOR I:=1 STEP 1 UNTIL N DO
         SE[I]:=SD[I]/SQRT(M)  ;
      WRITE (PTRO2,FOUT1,COUT) ;
      WRITE(PTRO2,FOUT2) ;
      FOR I:=1 STEP 1 UNTIL N DO WRITE(PTRO2,FOUT3,LST3)  ;
      GO TO L2 ;
   L1:CLOSE(CRDO2,RELEASE) ;
END.
```

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