



8-1982

Temporal Pattern of Gluconeogenesis in Diabetic and Non-Diabetic Chinese Hamsters

John P. Ofenstein

Follow this and additional works at: https://scholarworks.wmich.edu/masters_theses



Part of the Veterinary Medicine Commons

Recommended Citation

Ofenstein, John P., "Temporal Pattern of Gluconeogenesis in Diabetic and Non-Diabetic Chinese Hamsters" (1982). *Master's Theses*. 1717.

https://scholarworks.wmich.edu/masters_theses/1717

This Masters Thesis-Open Access is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in Master's Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact wmu-scholarworks@wmich.edu.



**TEMPORAL PATTERN OF GLUCONEOGENESIS
IN DIABETIC AND NON-DIABETIC CHINESE HAMSTERS**

by

John P. Ofenstein

**A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Science
Department of Biomedical Sciences**

**Western Michigan University
Kalamazoo, Michigan
August 1982**

TEMPORAL PATTERNS OF GLUCONEOGENESIS
IN DIABETIC AND NON-DIABETIC CHINESE HAMSTERS

John P. Ofenstein, M.S.

Western Michigan University, 1982

Gluconeogenesis is a normal physiological response of an individual to low blood glucose levels by which proteins are utilized in order to synthesize new glucose. Gluconeogenic rates in diabetic and normal Chinese hamsters were measured, on four different occasions over a 24 hour period, by injecting a radio-labelled precursor (^{14}C -lactate) into the animals. Then blood was tested for incorporation of the label into glucose. In the fed state, gluconeogenesis shows a circadian change in non-diabetic hamsters as well as in diabetic hamsters, with the diabetic hamsters showing a significantly greater gluconeogenic rate than the non-diabetic hamsters for every time point measured.

ACKNOWLEDGEMENTS

I am grateful to the Awards and Fellowships Committee at The Graduate College of Western Michigan University for financial assistance and to Dr. George Gerritsen of The Upjohn Company for supplying the Chinese hamsters. I am also greatly indebted to Dr. Albert Chang. of The Upjohn Company for his guidance and support throughout this project and to his assistant, B. J. Gilchrist, for performing the glucose and lactate assays.

The research and writing of this thesis could not have materialized were it not for the help I received from the personnel in the Biomedical Sciences Department at Western Michigan University. Especial thanks to my colleagues Karen Jodelis and Karen Ailsworth whose friendship and assistance during the actual experiment will not be forgotten; and to the members of my advisory committee, Dr. Leonard Beuving, Dr. Leonard Ginsberg, and Dr. Cecil McIntire, whose patience and encouragement have made the completion of this thesis and my graduation a reality. Finally, I sincerely thank Cathy Groat and Sue McIntire for handling the seemingly impossible task of typing this manuscript from my handwriting.

John P. Ofenstein

INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.
2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.
3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.
4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.
5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

**University
Microfilms
International**

300 N. Zeeb Road
Ann Arbor, MI 48106

1319735

OFENSTEIN, JOHN P.

TEMPORAL PATTERN OF GLUCONEOGENESIS IN DIABETIC AND NON-DIABETIC
CHINESE HAMSTERS

WESTERN MICHIGAN UNIVERSITY

M.S. 1982

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.	ii
LIST OF TABLES.	iv
LIST OF FIGURES	v
Chapter	
I. INTRODUCTION	
Explanation of Gluconeogenesis.	1
Literature Review: Gluconeogenesis	2
Purpose	6
II. MATERIALS AND METHODS	
Animal Models	7
<u>In Vivo</u> Gluconeogenesis Assay	7
Blood Glucose and Blood Lactate Measurements.	9
Total Radioactivity Measurements.	9
Statistics.	9
III. RESULTS	
Temporal Patterns of Gluconeogenesis.	13
Fluctuations in Blood Glucose	14
Fluctuations in Blood Lactate	15
Fluctuations in Total Radioactivity	15
IV. DISCUSSION.	21
V. CONCLUSION.	26
BIBLIOGRAPHY.	27

LIST OF TABLES

I. Experimental Procedure Outline.	11
--	----

LIST OF FIGURES

1. Rate of Gluconeogenesis.17
2. Blood Glucose Levels18
3. Blood Lactate Levels19
4. Blood Total Radioactivity,20

CHAPTER I

INTRODUCTION

Despite large fluctuations in metabolism, food intake, and physical activity, the concentration of glucose in the blood is maintained at a relatively constant level providing uninterrupted nourishment for the body tissues. The liver and kidneys of mammals are largely responsible for this glucose homeostasis. The liver possesses the capacity to store glucose from dietary carbohydrates in the form of glycogen and to breakdown a variety of substrates into molecules containing five or fewer carbon atoms that serve as precursors for carbohydrate synthesis. This latter process is known as gluconeogenesis and is a normal physiological response of an individual to the postprandial state. Gluconeogenesis occurs at all times but its rate will change in response to the body's need for glucose. The rate of gluconeogenesis will decrease with an increase of carbohydrates from the diet (Hanson, 1976) and after treatment with insulin (Gerritsen and Blanks, 1974; Wagle, 1976). Glucose is preferentially oxidized as a body fuel, stored as glycogen or converted to lipid for deposition in adipose tissue (Hanson, 1976). This decreased rate of gluconeogenesis is a normal response by the liver to the absorptive state during which time blood levels of insulin are increased and uptake of amino acids by the liver is decreased. The rate of gluconeogenesis will increase during periods of short term starvation, (Cherrington, Williams, Shulman and Lacy, 1981; Felig, Wahren,

Sherwin, Pulaiolagos, 1977; Wagle, Ingebretsen and Sampson, 1975), after treatment with glucocorticoids (Dunn, Chenowath and Bever, 1977); Exton, 1979; Exton, Harper, Tucker, Flagg and Park, 1973; Munck and Koritz, 1962), and in diabetes mellitus (Chang and Schneider, 1970; Exton, 1972; Felig and Sherwin, 1976; Friedman, Goodman and Weinhouse, 1965). The liver's response to the post-absorptive state is increased glucose production by means of glycogenolysis, the conversion of glycogen to glucose, and gluconeogenesis. Hepatic glycogen supplies the bulk of glucose released during brief fasts. As fasting is extended gluconeogenesis is augmented and glycogenolysis is diminished because of depletion of glycogen stores,

The attention of many researchers has focused on the role the liver plays in the aggravation of the diabetic syndrome. Experimental evidence both in vitro and in vivo have confirmed an increase in gluconeogenesis in the diabetic rat (Exton et al., 1973; Wagle et al., 1975), Chinese hamster (Chang and Schneider, 1970) and man (Demeutter and Shreeve, 1963; Hanson, 1976). Detailed studies describing glucose production from gluconeogenesis as related to the abnormal glucose homeostasis in the diabetic are very few and sometimes contradictory. Much of the controversy is over the relative importance of enhanced gluconeogenesis and of impaired peripheral glucose utilization in the pathogenesis of diabetes. Some of the confusion comes from the use of a variety of animal models because disparate results may be species specific rather than a consequence of the diabetic syndrome.

The Chinese hamster (*Cricetus griseus*) has been used as an experimental model in diabetic research for years (Chang, Noble, and Wyse, 1977; Gerritsen, Schultz and Johnson, 1973) resulting in considerable information describing particular strains with genetically spontaneous diabetes (Gerritsen, Johnson, Soret and Schultz, 1974) possessing metabolic and hormonal abnormalities similar to those found in man (Chang, 1970, Diani, Gerritsen, Stramsta and Murry, 1976; Gerritsen and Dulin, 1976).

The research into the cause of the increased gluconeogenic rate in diabetics has centered around the enzyme phosphoenolpyruvate carboxykinase (PEP-CK), the rate limiting enzyme in the gluconeogenic pathway (Chang, 1981; Exton, 1972; Hanson, 1976; Stalmans, 1976). During elevated rates of gluconeogenesis, the activity and concentration of PEP-CK in the cytosol of the liver cells is increased in both diabetic and non-diabetic hamsters in vivo (Chang, 1981; Chang et al., 1977; Chang and Schneider, 1970; MacDonald and Lardy, 1978) and in vitro (Chang and Schneider, 1970). Furthermore, in vivo studies on rats using inhibitors of PEP-CK have demonstrated a decrease in gluconeogenesis (Blackshear, Holloway and Alberti, 1975; MacDonald, 1980). Finally, the concentration of liver PEP-CK in the diabetic hamster (Chang, 1981; Chang et al., 1977), rat (Lardy, Faster and Young, 1966; Hanson, 1976), and rabbit (Huibregtse, Brunsvold, and Ray, 1976) is greater than the PEP-CK concentration in the non-diabetic animal. Much less information is available explaining the control of PEP-CK. However, studies have shown that as the

concentration of PEP-CK increases in the cytosol, the activity of PEP-CK also increases (Hanson, 1976). The question of whether the synthesis of PEP-CK is initiated by steroids or by the amount of substrate present is one that has yet to be answered.

Although glucocorticoids are known to influence gluconeogenesis, the detailed mechanism of their action remains to be elaborated. In vivo and in vitro studies in both normal and diabetic animals point toward direct stimulation of gluconeogenic enzymes (Exton, 1972; Exton et al., 1973; Haynes, 1962, Landau, Mahler, Ashmore, Elwyne, Hastings and Zottu, 1962; Munck and Koritz, 1962). The data has shown a decrease in gluconeogenesis following adrenalectomy and a return to normal gluconeogenesis following glucocorticoid therapy.

In work done by Woody (1980), the morning and evening levels of cortisol were measured in the normal and the diabetic Chinese hamsters. It was found that diabetic Chinese hamsters maintained plasma cortisol concentrations similar to those of controls for the morning and evening times examined. Similar studies have found that in vitro there is no alteration in the rate of gluconeogenesis following in vivo treatment with varying glucocorticoid concentrations so long as there is an adequate supply of gluconeogenic substrates in the culture medium (Dunn, Chenowath and Bever, 1977; Eisenstein, Spencer, Flatness and Bradsky, 1966; Smith and Long, 1967). These results point toward glucocorticoids as an indirect influence controlling the supply of gluconeogenic precursors to the liver.

Glucocorticoids are known to have the opposite effects on the rate of gluconeogenesis as compared with insulin, a suppressor of PEP-CK (Krahl, 1974). The blood levels of insulin in the diabetic hamster are lower than the insulin levels in the non-diabetic hamster during feeding (Gerritsen et al., 1973; Gerritsen et al., 1974; VanSickle, Gerritsen and Beuving, 1981). While in the fasted state, the insulin levels in both diabetic and non-diabetic hamsters decrease and the blood levels of the glucocorticoids increase (Exton, 1979). These glucocorticoid and insulin changes are associated with an overall increase in the rate of gluconeogenesis during fasting.

Fasted mice (Chang and Schneider, 1970), rats (Chang and Schneider, 1970; Exton, Corbin and Harper, 1972) and both normal and diabetic Chinese hamsters (Chang, 1981; Gerritsen and Dulin, 1967) have demonstrated increases in PEP-CK activity and gluconeogenesis. Also, because the length of time an animal is fasted affects these rates, measurements are usually taken only at one time of day. The results from these studies have shown the rate of gluconeogenesis in the fasted diabetic is greater than the rate in the fasted non-diabetic (Chan, Young, Hutson, Brumleg and Exton, 1975; Huibregtse, Brunsvold and Ray, 1976).

Dietary influences in the diabetic have also been investigated. Studies on the feeding habits of Chinese hamsters by Gerritsen and Blanks (1974) have demonstrated that the diabetic Chinese hamster consumes more food and water over a twenty-four hour period than does the non-diabetic Chinese hamster, but has a net retention of calories similar to the normal hamster. Other work in this area aimed at

limiting the caloric intake of pre-diabetic hamsters has shown a decrease in the onset and severity of diabetes (Dulin and Gerritsen, 1972; Gerritsen, Blanks, Miller and Dulin, 1974). VanSickle (1981) looked at the feeding habits of the Chinese hamster, at three hour intervals, over a twenty-four hour period and was able to establish that the pattern of food intake was similar in both diabetics and non-diabetics, with the diabetics consuming slightly more food than the non-diabetics at each interval.

More recently, studies have measured PEP-CK activity in the liver of the non-diabetic, fed rats and have shown the existence of a circadian rhythm (Kida, Nishio, Yokozawa, Nagai, Matsudu and Nakagawa, 1980), which parallels a similar rhythm in the rate of gluconeogenesis in these animals. Can this biological rhythm be demonstrated in the non-fasted Chinese hamster and the diabetic Chinese hamster?

The purpose of the present study was to measure the rate of gluconeogenesis at four different times during a twenty-four hour period in the non-diabetic and diabetic Chinese hamster. In an effort to minimize the exaggerated effects that fasting has on the glucocorticoid/insulin ratio and the overall rate of gluconeogenesis, all measurements were performed on animals fed ad libitum.

CHAPTER II

MATERIALS AND METHODS

Animals

Animals were obtained from the Chinese hamster colony of The Upjohn Company (Gerritsen and Blanks, 1974). Diabetic hamsters were selected from the ACHAC, ACHH, and ACAH sublines on the basis of a consistent Tes-Tape^R value of 4+ as determined by the Upjohn Company. Diabetic animals were matched with non-diabetic animals of the same age and sex from sublines AAM, AA, MAAA and MAA. All animals were maintained on Purina Mouse Breeder Chow and tap water ad libitum, and housed individually in a temperature (20° C) and humidity-controlled room. An alternating light/dark cycle was regulated with lights on at 0700 hr. and off at 1900 hr.

In Vivo Gluconeogenesis

The assay, as designed by Chang (1970) and modified by Chang (1980), was used to determine the blood levels of ¹⁴C-glucose following injection of ¹⁴C-lactate. A tracer amount of 2μC ¹⁴C(U)-lactate in 0.2 ml saline (165.2 m C/m mole, New England Nuclear) was injected subcutaneously. The maximum rate of incorporation of label into ¹⁴C-glucose in the blood occurred 15 minutes after injection. At this time 0.2 ml blood was collected from the orbital sinus by means of a 32 x 0.5 mm heparinized capillary tube.

Immediately upon removal of 0.2 ml blood, an 0.1 ml aliquot of blood was denatured by mixing it with 0.2 ml H_2O and heating at $100^{\circ}C$ for 1.5 minutes. After cooling, 0.5 ml chloroform was added in order to extract lipids. Two 75 μ l aliquots were removed from the aqueous supernatant and put into separate test tubes containing 12 μ l of 0.1 M acetic acid. One set of test tubes (blanks) received 0.15 ml H_2O and a second set (treated) received 0.15 ml glucose oxidase (Sigma Co.) which catalyzes the oxidation of glucose to gluconic acid. Both sets of test tubes were then treated in the same manner for the entire assay beginning with the addition of 0.265 ml of 0.05 M sodium acetate, pH 5.1, which had been gassed at room temperature with a mixture of 95% O_2 - 5% CO_2 for 15 minutes or longer. Samples were then gassed with the same gas mixture, capped and incubated at $37^{\circ}C$.

After 1.5 hours, 0.5 ml H_2O was added to all the test tubes and the resulting mixture was passed through an anion exchange column. Columns were prepared using 5-3/4 in. long Pasteur pipets, packed with QAE-Sephadex A-25 (Pharmacia) in 0.1 M ammonium formate, pH 7.4, which was held in place by glass wool. The gluconic acid from the samples treated with glucose oxidase was retained by this resin.

The columns were then washed four times with 1.0 ml H_2O . The eluant from each was collected into a vial containing a cation exchange resin consisting of 1.4 g Dowex Ag 50 WX4 (Bio-Rad, 100-200 mesh) in 0.05 M HCl. The vials were shaken at room temperature at 180 oscillations/min. for 5 minutes. Then 4.0 ml of supernatant were removed and introduced into counting vials containing 15 ml of Monophase 40 scintillation fluid (Packard). All vials were shaken and

counted in an Isocap/300 (Searle Model No. 6868) liquid scintillation counter. Counting efficiency was determined by using assay modified, nonlabelled hamster blood as the quenching agent. The difference in radioactivity present in the blank samples and the treated samples represented the amount of ^{14}C -glucose in 25 μl of blood.

Blood Glucose and Blood Lactate Measurement

In order to measure the pool size of both glucose and lactate in the blood at the time of gluconeogenesis measurement, a 20 μl aliquot of whole blood was mixed with 1.0 ml of 0.5N perchloric acid and frozen. After samples were thawed and centrifuged, the supernatant was used to determine glucose and lactate levels on an Auto Analyzer (Hoffman, 1937) at The Upjohn Company using the enzymatic fluorometric continuous-flow method (Lloyd, Burrin, Smythe and Alberti, 1978).

Total Radioactive Counts

A 50 μl aliquot of whole blood was collected into a 250 μl heparinized capillary tube and frozen. All samples were counted on the same day in 5 ml monophase 40 scintillation fluid.

Statistics

Prepared statistical packages of SAS were used to analyze data. A paired t-test was used in the determination of statistical significance between two means from the same group of animals. The standard t-test was used in all other correlation determinations with p 0.05 being significant and with p 0.1 being marginally significant.

Sequence of Procedures

Upon arrival, the hamsters were weighed and separated into two groups. Group I consisted of eight non-diabetic and eight diabetic hamsters. Group II consisted of another set of eight non-diabetic and eight diabetic hamsters. An entire group of 16 animals was used each time the rate of gluconeogenesis was measured (0700 hr., 1300 hr., 1600 hr., 0100 hr.). Only one group at a predetermined time was run through the assay each Saturday for four consecutive weekends allowing for at least two weeks recuperation period between bleedings (Table 1).

In an effort to minimize the stress of drawing blood from the animal, an acclimation period of two weeks was allowed. During this time the hamsters were removed from their cages at planned intervals and handled in the same manner they would be handled in the actual bleeding. The technique of drawing blood within seconds after removal of an animal from its cage was practiced using hamsters that were not included in the experimental groups. These same practice animals were used to determine that hematocrit recovered and that ^{14}C -lactate was removed from the hamsters' circulation in one week.

The procedure for measuring the rate of gluconeogenesis was as follows: eight hamsters, four diabetic and four non-diabetic, were brought into an adjacent treatment room. The animals were injected subcutaneously with 2 μl of ^{14}C -lactate in 0.2 ml saline at two minute intervals. After 15 minutes, 25-30 drops of blood were drawn from the orbital sinus via a 5 μl heparanized capillary tube, which emptied directly into a blood collecting dish. An aliquot of 0.1 ml blood

TABLE I

EXPERIMENTAL PROCEDURE OUTLINE

<u>Week</u>	<u>Time</u>	<u>Procedure</u>
I & II	Acclimation period	Animals were handled at different time periods.
III	1600 hr.	Group II - Blood was drawn and a gluconeogenesis assay performed. Blood for glucose and lactate measurements and total radioactivity was frozen.
IV	0700 hr.	Group I - Blood was drawn and a gluconeogenesis assay performed. Blood for glucose and lactate measurement and total radioactivity was frozen.
V	0100 hr.	Group II - Blood was drawn and a gluconeogenesis assay performed. Blood for glucose and lactate measurements and total radioactivity was frozen.
VI	1300 hr.	Group I - Blood was drawn and a gluconeogenesis assay performed. Blood for glucose and lactate measurement and total radioactivity was frozen.

was mixed in 0.2 ml H_2O and the solution was immediately immersed in a $100^{\circ}C$ water bath for 1.5 minutes and then cooled in an ice bath for future use in the gluconeogenesis assay. Another aliquot of 20 μ l was mixed with 1.0 ml perchloric acid and frozen for later measurement of glucose and lactate. The remaining blood was drawn into a heparinized pipet and frozen for later measurement of total radioactivity. The procedure was then repeated on the remaining eight hamsters in the group.

CHAPTER III

RESULTS

Temporal Patterns of Gluconeogenesis

In the non-diabetic Chinese hamster the maximum rate of gluconeogenesis as measured by incorporation of ^{14}C -lactate into glucose at four different time periods (0700, 1300, 1600, 0100 hrs.) over 24 hours, occurred at 0100 hrs. It was significantly different from the minimum rate occurring at 1600 hrs. Measurements of gluconeogenesis taken later in the morning and in the afternoon showed a progressive decrease of 59% between 0700 hr. and 1600 hr. (Figure 1).

The diabetic Chinese hamster appeared to follow the same temporal pattern in gluconeogenic rate with the exception of the measurement between 1300 hr. and 1600 hr. During this time period the diabetics had an increase in gluconeogenesis while the non-diabetic had a decrease which was non-significant in both types of animals (Figure 1).

In the diabetic Chinese hamster the maximum rate of gluconeogenesis also occurred at 0100 hr. This was marginally significant from the minimum rate at 1300 hr. The diabetic also showed later morning and early afternoon decreases in gluconeogenesis of 13% at 0700 hr. and 28% at 0300 hr. similar to those experienced by the non-diabetic during the same time period. However, the minimum rate of gluconeogenesis occurred earlier in the diabetic than in the non-diabetic, after which time the diabetic experienced increases of

14.9% at 1600 hr. and 38.1% at 0100 hr, as seen in Figure 2 with a marginal significance of $p = 0.0574$ between the measurements at 1600 hr. and 0100 hr.

The rate of gluconeogenesis in the diabetic Chinese hamster was significantly greater than the rates of gluconeogenesis in the non-diabetic for every time period tested (Figure 2).

Fluctuations in Glucose

In the non-diabetic Chinese hamster the maximum amount of glucose in the blood occurred at 1600 hr. and was found to be significantly different from the minimum value at 1300 hr. (Figure 2). Glucose levels in the blood fluctuate very little during the evening hours, measuring a small decrease of 11% followed by an even smaller non-significant increase of 2% at 0700 hr. Only in the early afternoon measurement do glucose levels show a significant decrease of 19% followed by a significant increase of 37% (Figure 2).

In the diabetic Chinese hamster the maximum amount of blood glucose was also measured at 1600 hr. However, in the diabetic a significant decrease of 23.7% can be seen during the evening hours, after which glucose levels progressively increased from the recorded minimum at 0100 hr. for the next three measurements (Figure 2).

The amount of blood glucose in diabetic Chinese hamsters was significantly greater than the blood glucose of the non-diabetic for every time period tested (Figure 2).

Fluctuations in Lactate Total

In the non-diabetic Chinese hamster the maximum amount of lactate in the blood was measured at 1600 hr. and was found to be significantly different from the minimum value at 1300 hr. (Figure 3). Lactate levels in the blood showed a significant decrease during only the early afternoon which was at the same time as the only significant decrease in glucose levels in the blood were recorded.

The temporal pattern of the diabetic's blood lactate fluctuations were very similar to the non-diabetic's. In the diabetic, maximum blood lactate was measured at 0100 hr. and was found to be significantly different from the minimum value at 1300 hr. (Figure 3). The diabetic experienced a similar decrease in lactate levels in the blood from 0700 hr. to 1300 hr. of 36% as compared with the non-diabetic's 39% decrease during this same time period. The following 49% increase in the blood lactate made the diabetic's pattern of lactate fluctuations very similar to those of the non-diabetic's. However, the amount of blood lactate found in the diabetic Chinese hamster was consistently lower than the non-diabetic for every time period measured (Figure 3).

Fluctuations in Radioactivity

In the non-diabetic Chinese hamster the maximum amount of radioactivity measured in the blood, among the four time periods, occurred at 1300 hr. and was significantly different from the minimum amount of radioactivity measured at 0100 hr. (Figure 4). It is during this maximum total radioactivity that the specific activity of lactate is the greatest in the non-diabetic and diabetics. The

maximum total radioactivity in the diabetic also occurred at 1300 hr, and was significantly different from the minimum radioactivity at 1600 hr.

The amount of radioactivity in the diabetic was significantly greater than the amount found in the non-diabetic for every time period measured (Figure 4). However, when the total radioactivity was corrected for ^{14}C -glucose counts in both diabetic and non-diabetic this difference between the two disappeared with only one time period, at 1300 hr., showing a significant difference.

RATE OF GLUCONEOGENESIS

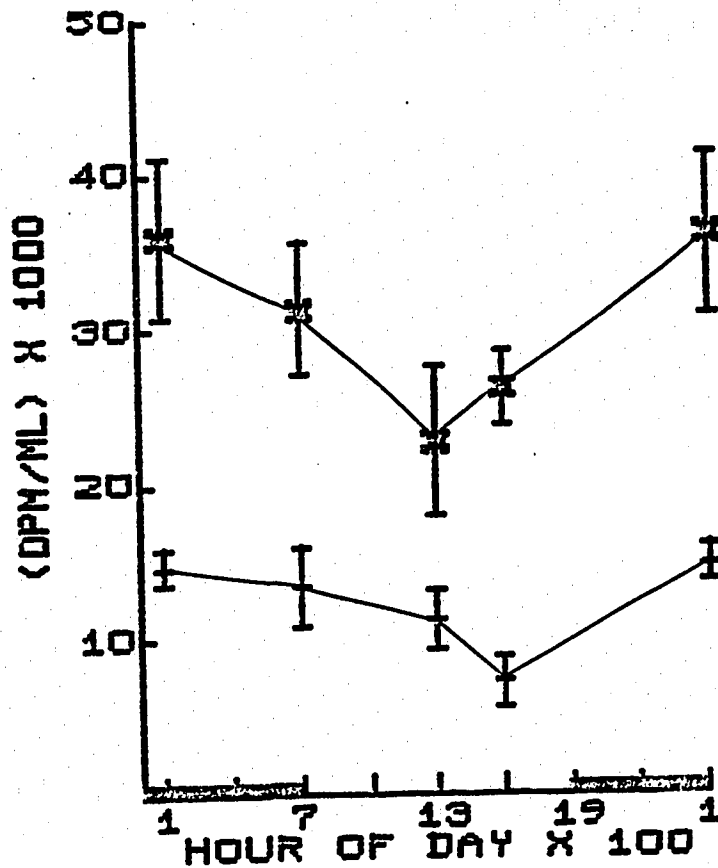


Figure 1: Plot of mean ^{14}C -glucose * hour of day. \pm SEM
 x = diabetics and + = non-diabetics.
 Diabetic max. = 0100 hr. and min. = 1300 hr. $p < 0.05$
 Non-diabetic max. = 1600 hr. and min. = 1300 hr. $p < 0.05$
 Between diabetics and non-diabetics for all measurements
 $p < 0.05$

BLOOD GLUCOSE LEVELS

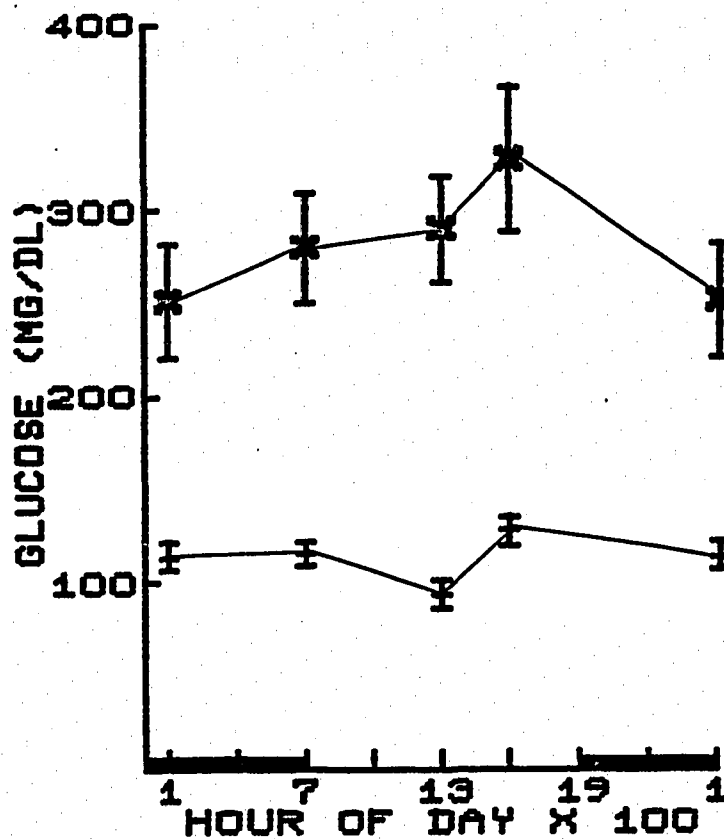


Figure 2: Plot of mean glucose * hour of day. \pm SEM,
 x = diabetics and + = non-diabetics,
 Diabetic max. = 1600 hr, and min. = 0100 hr., $p < 0.05$
 Non-diabetic max. = 1600 hr, and min. = 1300 hr., $p < 0.05$
 Between diabetic and non-diabetic for all measurements
 $p < 0.05$

BLOOD LACTATE LEVELS

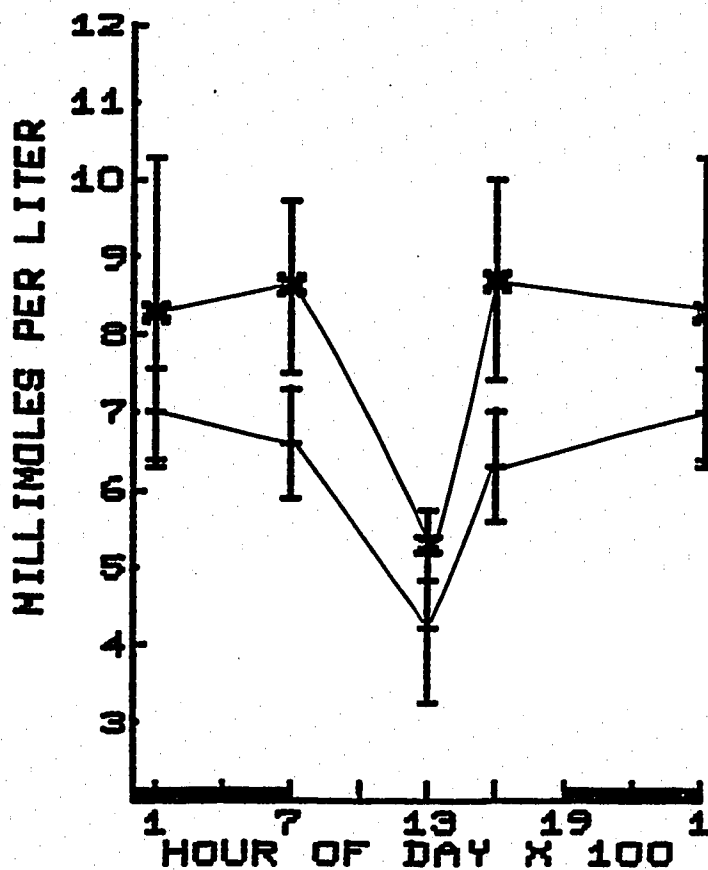


Figure 3: Plot of mean lactate * hour of day, \pm SEM.
 x = diabetic and + = non-diabetic,
 Diabetic max, = 0100 hr. and min, = 1300 hr., $p < 0.05$
 Non-diabetic max. = 0100 hr, and min. = 1300 hr., $p < 0.05$

BLOOD TOTAL RADIOACTIVITY

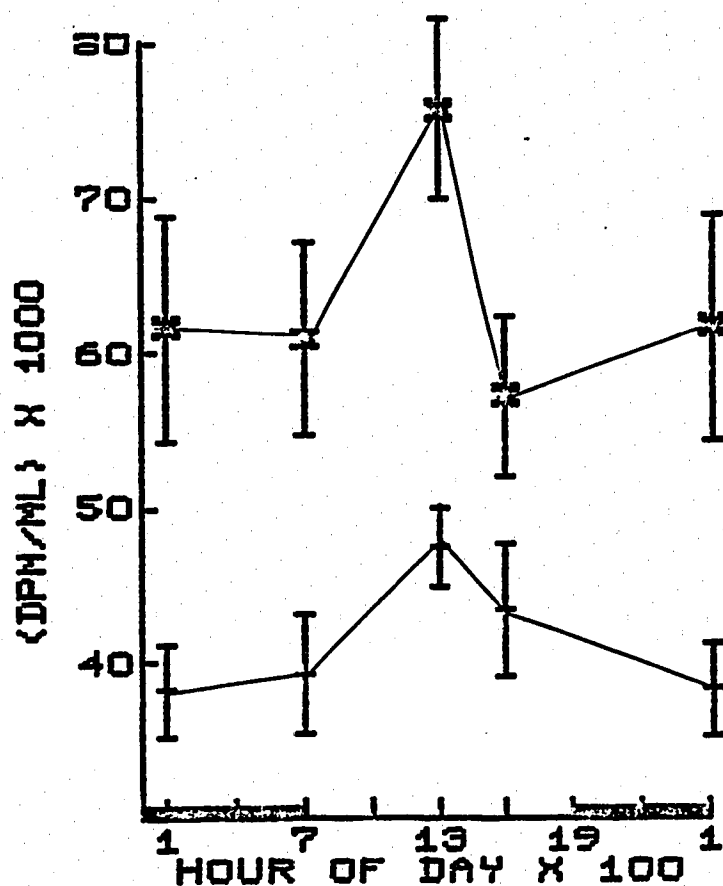


Figure 4: Plot of mean total radioactivity * hour of day, \pm SEM.
 x = diabetics and + = non-diabetics
 Diabetic max, = 1300 hr, and min. = 1600 hr., $p < 0.05$
 Non-diabetic max, = 1300 hr. and min. = 0100 hr, $p < 0.05$
 Between diabetic and non-diabetic for all measurements $p < 0.05$

CHAPTER IV

DISCUSSION

The rate of gluconeogenesis in the non-diabetic Chinese hamster during the fed state exhibits a circadian rhythm consistent with the previously reported activity of PEP-CK and rates of gluconeogenesis in fed rats (Kida et al., 1980). In this study, the maximum rate of gluconeogenesis occurred during the animals' most active period (while the lights were off, from 0700 hr. to 1900 hr.). These gluconeogenic measurements correlate with Kida's et al. (1980) results which showed maximum activity of PEP-CK occurred during the evening at 2000 hr. and a minimum at 0800 hr. This study, therefore, indirectly supports the argument that PEP-CK is a rate-limiting enzyme for gluconeogenesis.

The particular time periods chosen to measure gluconeogenic rates were based on the work done by Van Sickle et al. (1981) on food consumption in the Chinese hamster. The results obtained in the present study correlate positively with Van Sickle's findings, showing that the maximum food intake, by both diabetic and non-diabetic Chinese hamsters, occurred at the end of their active period (0700 hr.) and that the minimum food intake occurred during their non-active period (1600 hr.).

In comparison with the previous work done on fasted animals, the results of the present study on fed animals indicate that gluconeogenic rates gradually decreased from a maximum in the evening to

a minimum in the afternoon. These findings are consistent with Woody's (1980) results which described gluconeogenic rates in fasted normal Chinese hamsters as being greater in the evening than in the morning.

The non-diabetic Chinese hamster's temporal pattern of gluconeogenesis can be accounted for by the animal's nocturnal habits. As the food intake of the animal decreases throughout the morning hours and into the afternoon, the metabolic rate begins to slow down. Normally the liver obtains lactate for use in glucose synthesis from the glycolysis pathway. As the animal's metabolic rate slows, the amount of lactate available for gluconeogenesis becomes less (Figure 3) causing the rate of gluconeogenesis to slow down. During the hamster's daylight non-active period when the rate of gluconeogenesis is slowing down and food consumption is at a minimum, the glucose level in the blood also begins to drop (Figure 2). The decrease in the rate of gluconeogenesis and blood lactate could account for the increase in total radioactivity found in the blood during the afternoon hours (Figure 4). Similarly, the decrease in food consumption and gluconeogenesis could account for the decrease in blood glucose during the same period (Figure 2).

It is possible that the animal's liver responds to the low blood glucose by increasing glycogenolysis which causes a sharp increase in blood glucose levels in the late afternoon and maintains this glucose level until PEP-CK activity increases (Kida et al., 1980). Acceleration of PEP-CK enzymatic activity causes an increase in the rate of gluconeogenesis which will maintain the blood glucose

level as the stored glycogen is being depleted. Finally, as food intake increases during the dark phase, the PEP-CK level decreases and the circadian rhythm continues. In contradistinction, the PEP-CK activity in the fasted animal remains high in order to maintain the blood glucose level via gluconeogenesis. Previous studies on fasted diabetic Chinese hamsters have used radiolabeled precursors to gluconeogenesis such as pyruvate (Chang et al., 1970; Woody, 1980) to study gluconeogenic rates. In the present study radiolabeled lactate was chosen because it has been shown to be the main substrate for production of blood glucose in the liver (Kida et al., 1980). Because of the different radiolabeled precursors used in fasted studies and in this study, it can only be speculated that base line rates for gluconeogenesis are decreased during the fed state because of the lower ^{14}C -glucose counts measured.

The diabetic Chinese hamster in the fed state can be compared to a starving animal, in that PEP-CK activity is greater (Chang, 1970) and that the rate of gluconeogenesis is two to three times greater than the rate of gluconeogenesis in the fed non-diabetic animal. In Van Sickle's et al. (1981) results, the diabetic hamster's feeding times were reported to follow a similar pattern to that of the non-diabetic animal. Although the diabetic hamster consumed more food at each feeding, it had a lower net caloric retention than the non-diabetic due to caloric loss as glucose in the urine (Gerritsen and Blanks, 1974) and as other materials in the feces possibly due to abnormalities in the small intestine (Dianai and Gerritsen, 1976). This caloric loss could account for the lack of obesity in the

diabetic animals. Results of the present study suggest that the diabetic hamster's food consumption or caloric intake affects the rate of gluconeogenesis. As food consumption increases, rates of gluconeogenesis decrease and vice versa,

Data from the present study supports the existence of a circadian rhythm in the diabetic hamster which is similar in temporal patterns to the rhythm found in the non-diabetic hamster, but with the diabetic experiencing a greater rate of gluconeogenesis for every time period measured. The temporal patterns of gluconeogenesis and blood glucose levels in the diabetic hamster tend to fluctuate more dramatically and earlier in the day than in the non-diabetic hamster, suggesting the diabetic hamster might be experiencing an overall faster onset of increased gluconeogenesis. At the same time period the diabetic hamsters reached their minimum rate of gluconeogenesis, their blood lactate levels also reached a minimum. The non-diabetic hamster's blood lactate levels reached a minimum at the same time period as the diabetic hamster, even though the non-diabetic's rate of gluconeogenesis had not yet reached a minimum (Figure 3). In the diabetic hamsters, the blood lactate levels were consistently lower than in the non-diabetics but not significantly lower. The lower blood lactate levels may be attributed to an increased rate of gluconeogenesis.

In the present study it was assumed that the specific activity of lactate was the same in both the non-diabetic and the diabetic hamsters. This assumption was based on the injection of the same amount of ^{14}C -lactate into both diabetic and non-diabetic animals and

on the similar blood lactate levels found in the diabetics and the non-diabetics. This also assumes both diabetic and non-diabetic hamsters absorbed the radiolabel into their blood at the same time.

The total radioactivity in the blood of the diabetic hamster was significantly higher than in the non-diabetic hamsters. This could be attributed to the excess amounts of labeled glucose found in the diabetic.

CHAPTER V

CONCLUSION

In conclusion, our findings indicate that, in the fed state, gluconeogenesis shows a circadian rhythm in the non-diabetic Chinese hamsters as well as in the diabetic Chinese hamsters. Both animals experienced a maximum rate of gluconeogenesis during the evening hours that was significantly different from the minimum rate of gluconeogenesis recorded during the afternoon. Furthermore, while in the fed state, the diabetic Chinese hamster showed a significantly greater rate of gluconeogenesis than in the non-diabetic Chinese hamster for every time point measured.

BIBLIOGRAPHY

- Blackshear, P.J., Holloway, P.A., & Aberti, K.G., The effects of inhibition of gluconeogenesis on ketogenesis in starved and diabetic rats. Biochem J., 1975, 148 (3), 353-362.
- Chan, T.M., Young, K.M., Hutson, N.J., Brumley, F.T. & Exton, J.H. Hepatic metabolism of genetically diabetic mice. I. Carbohydrate metabolism. Am J. Physiol., 1975, 229, (6), 1702-1712.
- Chang, A.Y. Biochemical abnormalities in the Chinese hamster (*Cricetulus Griseus*) with spontaneous diabetes. Int J. Biochem., 1981, 13 (1), 41-3.
- Chang, A.Y. Insulin synthesis and secretion by isolated islets of spontaneously diabetic Chinese hamsters. In: The structure and metabolism of the pancreatic islets. Wenner-Gren Symposium No. 6. Oxford, New York, Pergamon Press, 1970, 515-526.
- Chang, A.Y., Noble, R.E., & Wyse, B.M. Streptozotocin-induced diabetes in the Chinese hamster. Biochemical and endocrine disorders. Diabetologia, 1977 13 (16), 595-602.
- Chang, A.Y., Noble, R.E. & Wyse, B.M. Comparison of highly inbred diabetic and nondiabetic lines in the Upjohn colony of Chinese hamsters. Diabetes, 1977, 26, 1063-1071.
- Chang, A.Y. & Schneider, D.I. Rate of gluconeogenesis and levels of gluconeogenic enzymes in liver and kidney of diabetic and normal Chinese hamsters. Biochem Biophys Acta, 1970, 222, 587-592.
- Chang, A.Y. & Schneider, D.I. Abnormalities in hepatic enzyme activities during development of diabetics in Db mice. Diabetologia, 1970, 6, 274-278.
- Chang, A.Y. & Schneider, D.I. Metabolic abnormalities in the pancreatic islets and livers of the diabetic Chinese hamsters. Diabetologia, 1970, 6, 180-185.
- Cherrington, A.D., Williams, P.E., Shulman, G.I., & Lacy, W.W. Differential time course of glucagon's effect on glycogenolysis and gluconeogenesis in the conscious dog. Diabetes, 1981, 30, 180-187.
- Demeutter, R.C. & Shreeve, W.W. Conversion of DL-Lactate-2¹⁴C or 3-¹⁴C or Pyruvate - 2 - ¹⁴C to blood glucose in humans: effects of diabetes, insulin, tolbutamide and glucose load. J. Clin Invest., 1963, 42, 525-533.

- Diani, A.R., Gerritsen, G.C., Stramsta, S., & Maurry, P. A study of morphological changes in the small intestine of the spontaneously diabetic Chinese hamster. Diabetologia, 1976, 12, 101-109.
- Dulin, W.E. & Gerritsen, G.C. Interaction of genetics and environment on diabetes in the Chinese hamster as compared with human and other diabetic species. Acta Diabet Lat, 1972, 9, (Suppl. 1), 48-84.
- Dunn, A., Chenoweth, M., & Bever, K. Use of ^3H and ^{14}C doubly labeled glucose and amino acids in the study of hormonal regulation of gluconeogenesis in rats. Fed. Proc, 1977, 36 (2), 245-252.
- Eisenstein, A., Spencer, S., Flatness, S., & Bradsky, A. Carbohydrate synthesis in the isolated perfused rat liver: Role of the adrenal cortex. Endocrinology, 1966, 79, 182-186.
- Exton, J.H. Gluconeogenesis. Metabolism, 1972, 21 (10), 945-990.
- Exton, J.H. Regulation of gluconeogenesis by glucocorticoids. Manager Endocrinol, 1979, 12, 535-546.
- Exton, J.H., Crobin, J.G., & Harper, S.C. Control of gluconeogenesis in liver. V. effects of fasting, diabetes and glucagon on lactate and endogenous metabolism in the perfused rat liver. J. Biol Chem, 1972, 25, 247 (16), 4996-5003.
- Exton, J.H., Harper, S.C., Tacker, A.L., Flagg, J.L., & Park, C.R. Effects of adrenalectomy and glucocorticoid replacement on gluconeogenesis in perfused livers from diabetic rats. Biochem Biophys Acta, 1973, 379, 41-57.
- Felig, P. Sherwin, R. Carbohydrate homeostasis, liver and diabetes. Prog Liver Dis, 1976, 5, 149-171.
- Friedman, B., Goodman, E.H., Jr. & Weinhouse, S. Dietary and hormonal effects on gluconeogenesis in the rat. J. Biol Chem, 1965, 240, 3729-3735.
- Gerritsen, G.C. & Blanks, M.C. Characterization of Chinese hamsters by metabolic balance, glucose tolerance and insulin secretion. Diabetologia, 1974, 10, 493-499.
- Gerritsen, G.C., Blanks, M.C., Miller, R.L. & Dulin, W.E. Effect of diet limitation on the development of diabetes in prediabetic Chinese hamsters. Diabetologia, 1974, 10, 559-565.
- Gerritsen, G.C., & Dulin, W.E. Characterization of diabetes in the Chinese hamster. Diabetologia, 1967, 3, 74-84.
- Gerritsen, G.C., Johnson, M.A., Soret, M.G. & Schultz, J.R. Epidemiology of Chinese hamsters and preliminary evidence for genetic heterogeneity of diabetes. Diabetologia, 1974, 10, 581-588.

- Gerritsen, G.C., Schultz, J.R. & Johnson, M.A. Preliminary analysis of incidence, onset of diabetes and survival rates of genetically fixed, reproducible population of Chinese hamsters. Diabetes, 1973, 22 (Suppl. 1), 307.
- Hanson, R.W. & Mehrlan, M.A. (Eds) Gluconeogenesis: Its regulation in mammalian species. New York: John Wiley & Sons, 1976, 533-555.
- Haynes, R. Studies of an in vitro effect of glucocorticoids on gluconeogenesis. Endocrinology, 1962, 71, 399-406.
- Hoffman, W.S. A rapid photoelectric method for the determination of glucose in blood and urine. J. Biol Chem, 1937, 120, 51-55.
- Huibregtse, C.A., Brunsvold, R.A. & Ray, P.D. Dietary and hormonal regulation of some enzyme activities. Biochem Biophys Acta, 1976, 24, 421 (2), 228-236.
- Kida, K., Nishio, T., Yokozawa, T., Nagai, K., Matsuda, H., & Nakagawa, H. The circadian change of gluconeogenesis in the liver. In vivo in fed rats. J. Biochem, 1980, 88, 1009-1013.
- Krahl, M. Endocrine functions of the pancreas. Annual Review of Physiology, 1974, 36, 331-360.
- Landau, B., Mahler, R., Ashmore, J., Elwyn, D., Hastings, A. & Zottu, S. Cortisone and the regulation of hepatic gluconeogenesis. Endocrinology, 1962, 70, 47.
- Lardy, H.A., Foster, D.O., Young, J.W., Shrago, E. & Ray, P.D. Hormonal control of enzymes participating in gluconeogenesis and lipogenesis. J. Cell and Comp Physiol, 1966, 66, 39-54.
- Lloyd, B., Burrin, J., Smythe, P. & Albertia, G.M.M. Enzymatic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. Clin Chem, 1978, 34, (10), 1724-1728.
- MacDonald, M.J. Rapid inactivation of rat liver. Phosphoenolpyruvate carboxykinase by microsomes and reversal by reductants. Biochem Biophys Acta, 1980, 615 (1), 223-236.
- MacDonald, M.J. & Lardy, H.A. 3-aminopicolinate: a synthetic metal-complexing activator of phosphoenolpyruvate carboxykinase. J. Biol Chem, 1978, 253 (7), 2300-2307.
- Maught, T.H. Hormone receptors: New clues to the cause of diabetes. Science, 1976, 193, 220-222.
- Munck, A. & Koritz, S.B. Studies on the mode of action of glucocorticoids in rats. Biochem Biophys Acta, 1962, 57, 310-317.

- Riley, V. Adaptation of orbital bleeding technic to rapid serial blood studies. Proc. Soc Exp Biol, 1960, 104, 751-754.
- Seitz, H.J., Kaiser, M., Krone, W. & Tarnowski, W. Physiologic significance of glucocorticoids and insulin in the regulation of hepatic gluconeogenesis during starvation in rats. Metabolism, 1976, 25, 1545-1555.
- Smith, D., & Long, C. Effect of cortisol on the plasma amino nitrogen of eviscerated adrenalectomized diabetic rats. Endocrinology, 1967, 80, 561-566.
- Stalman, W. The role of the liver in the homeostasis of blood glucose. Curr Top Cell Regul, 1976, 11, 51-97.
- Van Sickle, W.V., Gerritsen, G.C. & Beuving, L. A comparison of circadian rhythms in feeding, plasma insulin, glucose and glucagon between normal and diabetic Chinese hamsters. Chronobiologia, 1981, 8, 1-6.
- Wagle, S.R. Studies of hormonal regulation of metabolism using isolated hepatocytes. Acta Diabetol. Lat, 1976, 13 (5-6), 186-201.
- Wagle, S.R., Ingebretsen, W.R. & Sampson, L. Studies on gluconeogenesis and stimulation of glycogen and protein synthesis in isolated hepatocytes in alloxan diabetic, normal fed and fasted animals. Acta Diabetol Lat, 1975, 12, (3-47), 185-189.
- Woody, C. The effects of glucocorticoids on gluconeogenesis in diabetic and normal Chinese hamsters. (M.S. Thesis, Western Michigan University, 1980).
- Waitman, R., Shoemaker, W., & Larin, F. Proc. Nat. Acad. of Science, 1968, 59, 800-807.
- Wyse, B., & Dulin, W.E. Insulin and glucagon levels in the normal and spontaneously diabetic Chinese hamster. (Submitted for publication).
- Wyse, B.M. & Dulin, W.E. The influence of age and dietary conditions on diabetes in the Db mouse. Diabetologia, 1970, 6, 268-273.