A COMPARTMENTAL ANALYSIS OF THE PRODUCTION, INTERCONVERSION AND METABOLISM OF VOLATILE FATTY ACIDS BY THE RUMEN MICROBIAL ECOSYSTEM IN STEERS CONSUMING A WHOLE OR GROUND CORN HIGH-CONCENTRATE RATION

By

WILLIAM MICHAEL SHARP

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1970

Submitted to the Faculty of the Graduate College
of Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1977

A COMPARTMENTAL ANALYSIS OF THE PRODUCTION, INTERCONVERSION AND METABOLISM OF VOLATILE FATTY ACIDS BY THE RUMEN MICROBIAL ECOSYSTEM IN STEERS CONSUMING A WHOLE OR GROUND CORN HIGH-CONCENTRATE RATION

Thesis Approved:

F N Owens
Thesis Adviser

Donald G Wagner

Roger E. Koeppe

Robert J. Mullholland

Norman N Durham
Dean of the Graduate College

PREFACE

This dissertation is addressed to the study of volatile fatty acid production and interconversion in the rumen of steers consuming high-concentrate diets. Radioactive tracers and compartmental analysis are used in the development of specific models describing the movement of carbon through the ruminal VFA pools.

So many people have helped me throughout my academic career at Oklahoma State University that I hardly know where to begin. I am grateful to the Departments of Animal Science and Biochemistry for their generous financial support, which chiefly consisted of a graduate assistantship and the provision of excellent laboratory facilities, equipment and supplies.

I wish to express my sincere appreciation to Dr. R. R. Johnson, former Professor of Animal Science and Biochemistry, for his guidance and assistance during the initial stages of this study. I would also like to thank Dr. F. N. Owens, Associate Professor of Animal Science, for assuming the chairmanship of my graduate committee vacated when Dr. Johnson became Head of the Animal Science Department at The University of Tennessee. The many helpful discussions with and suggestions from Dr. Owens during the preparation of this thesis were greatly appreciated. A deep appreciation is extended to Dr. R. E. Koeppe, Professor and Head of Biochemistry, Dr. R. J. Mulholland, Professor of Electrical Engineering, Dr. H. O. Spivey, Professor of Biochemistry and Dr. D. G.

Wagner, Professor of Animal Science for their service on my committee and their helpful suggestions throughout the course of this study. I am especially indebted to Dr. Mulholland for his guidance in systems analysis, the many stimulating discussions concerning compartmental analysis techniques and his sincere interest in this project.

I would also like to thank Dr. R. L. Noble, Professor of Animal Science, for his friendship and encouragement as my lower division undergraduate advisor. Furthermore, a sincere appreciation is extended to Dr. A. D. Tillman, my upper division undergraduate advisor, for his advice and encouragement to enter graduate school.

I would like to thank Dr. F. R. Leach for the use of the liquid scintillation spectrometer, Dr. E. C. Nelson for the use of the fraction collector and Dr. G. V. Odell for the use of the preparative gas chromatograph. Grateful acknowledgement is also extended to Mr. Jim McGee, Jr. and the University Computer Center for their assistance and co-operation in obtaining the computer drawn graphs. I would also like to thank Dr. J. H. Thornton, post-doctoral fellow, for many helpful discussions during this study. Further appreciation for assistance is extended to other faculty members, fellow graduate students, secretaries, animal caretakers and laboratory technicians. Recognition is extended to Ms. Linda Good for her assistance with some of the gas chromatography analysis and Ms. Phyllis Sharp for her assistance during the sample collection phase. A special thanks is extended to Ms. Diane Wheeler for her valuable assistance with the silicic acid columns and in the preparation of many of the illustrative materials used in this thesis. I would also like to thank Ms. Sue Heil for her patience, advice and excellent typing of this manuscript.

I am deeply indebted to my mother and father, Pat and Bill Sharp, for their unselfish sacrifices in making it possible for me to go to college. Nearly 30 years ago they began a savings plan, founded upon and sustained by the sale of a few head of steers each year, which would enable their children to further their education beyond high school. The completion of this program undoubtly marks a major milestone in that dream. I am extremely grateful for the opportunities they have given me. I am also thankful for the encouragement and support from my sister, Sharon, and brothers, Richard and Philip, during this program of graduate study. A special thanks is extended to Richard for his invaluable assistance with some of the computer software used throughout this study. I would also like to thank my grandmother, Mrs. Eliza Bennett, for her unfailing confidence that I would finally finish this program.

Finally, I would like to dedicate this dissertation to the memory of my grandfather, Donald W. Bennett. Undoubtly his scientific curiosity coupled with his engineering background did much to influence the development of my current interests and attitudes. I will always remember the innumerable talks we would have ranging from meteorology to oil refining to bird watching.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

NOMENCLATURE

Mathematical Variables

$\underline{A}$ - Specific activity matrix for a complete compartmental system

a_{ik} - Specific activity of the i^{th} compartment during the k^{th} experiment expressed as units of tracer per unit weight of natural atoms of the same species

$\underline{A}^*$ - Specific activity matrix for an incomplete compartmental system

$\underline{A}^T$ - Transpose of $\underline{A}$

a' - Adjusted specific activity

$\underline{B}$ - Input connection matrix

CF - Correction factor to adjust for differences in metabolic size

D - Total quantity of tracer in a single dose

DR - Irreversible disposal rate

$\underline{E}$ - Matrix of error terms associated with terms in $\underline{M}$

EP - Effective production rate

$\underline{F}$ - Matrix of compartmental turnovers

f - Rate of transfer (flow) of unlabeled material; i.e., turnover rate. Units of mass per unit time.

f_{ii} - Sum of all flows of material out of compartment i

f_{ij} - Flow of material from compartment j to compartment i

$\underline{f}^*$ - Vector of flow rates for an incomplete compartmental system

g - Vector of eigenvalues corresponding to a compartmental system

$\underline{H}$ - Matrix of eigenvectors associated with a compartmental system

$\underline{I}$ - Identity matrix

$\underline{K}$ - Matrix of compartmental turnover rate constants

k_{ij} - Rate constant of transfer from pool j to pool i in terms of fraction of total content moving per unit time

$\mathcal{L}^{-1}$ - Inverse Laplace transform operator

$\underline{M}$ - Matrix of average specific activity

M_{fi} - Molar fraction of acid i

M_o - Marker concentration at t=0

P - Production rate of tracee; i.e., rate of entry of <u>new</u> tracee atoms into a compartment

$\underline{\Phi}$ - State transition matrix

Q - Quantity of unlabeled material (tracee) in a pool (compartment)

q - Quantity of tracer in a pool

RE - Relative error

SE - Standard error

s - Standard deviation

T - Turnover time. Time required for that quantity which is exactly equal to Q to move in and out

t - Time

$\underline{U}$ - Matrix of compartmental inputs (infusion rates) for a complete compartmental system

u - Input vector, infusion rate

u^* - Vector of infusion rates for an incomplete compartmental system

V_m - Volume of distribution for marker, m

W - Weight

x - State variable (general)

$\dot{x}$ - Time derivative of the state variable x

$\underline{X}(s)$ - Laplace transform of $\underline{x}(t)$

Y_{ji} - Transfer ratio. Fraction of j coming from i

z - Compartmental throughput

Subscripts

a - Acetate

b - Butyrate

c - Caproate

e - Equilibrium or steady-state value

g - Glucose

h - Hexose

m - Marker

o - Outside the system

p - Propionate

T - Total

v - Valerate

CHAPTER I

INTRODUCTION

The development of the American Beef System over the last century has been one of the most colorful and progressive eras in the history of American agriculture. Undoubtly consumer demand for beef at a reasonable price inspired Joseph McCoy to drive thousands of cattle from Texas to the railheads in Missouri and Kansas. In recent years, this same consumer demand for high quality beef at the lowest possible price has led to the development of the large commercial feedlots located throughout the High Plains area. Although corn and sorghum are the principle grains fed to these cattle, a multitude of grain processing methods have been utilized in an attempt to maximize the efficiency of beef production. These grain processing techniques include rolling, crimping, grinding, steam flaking, micronizing, popping, reconstituting, high-moisture harvesting and propionic acid preservation. Quite often corn is fed unprocessed; however, sorghum is generally processed by one or a combination of these methods due to the small size of the kernel and hardness of the seed coat.

Research at several universities and colleges and in the field has evaluated these processing methods on the basis of feed efficiency and rate of liveweight or carcass gain. In addition, some studies have measured the digestibility of various diets containing these processed grains. Since most of the carbohydrate in these high-

concentrate rations is in the form of starch, the digestibility of this component becomes a major consideration. In general, total starch digestibilities are similar, but the site of digestion may vary drastically (Hinmann and Johnson, 1974; Cole et al., 1976a; Galyean et al., 1976). For example, the starch in steam flaked corn is essentially completely degraded in the rumen. In contrast, considerable amounts of starch have been found flowing through the abomasum of steers consuming diets containing whole or ground shelled corn.

Measurements commonly used to assess the degree of rumen fermentation on these type of diets have generally involved the determination of pH, volatile fatty acid (VFA) concentrations and their relative molar proportions and, in some cases, lactic acid levels. Quite often VFA concentration has been used as an index describing the level or intensity of the fermentation, while the molar proportion of the various VFA's has been interpreted as a reflection of the relative rates of VFA production. Experiments conducted by Gray et al. (1966), Weller et al. (1967) and Weston and Hogan (1968) seem to support this hypothesis; however, these studies involved sheep consuming forage diets. These assumptions may not be valid for beef cattle consuming high-concentrate diets due to the drastic differences in the rumen ecosystem and the intensity of rumen fermentation observed with these diets. Research investigating the in vivo ruminal production of VFA's in beef cattle consuming high-concentrate diets has been very limited. Therefore, the purpose of this study was to investigate, using ^{14}C labeled VFA's, the production and interconversion rates of the major VFA's (acetic, propionic and butyric acids) in the rumen of steers

consuming a high-concentrate corn ration. The corn was fed in either the whole or ground form in an attempt to evaluate the effect of grain processing on the rates of VFA production and interconversion. Several studies have shown that when diets containing greater than 80% corn are fed to beef cattle, performance is generally higher when whole shelled corn is fed as compared to that obtained with ground corn (Vance et al., 1970; Vance et al., 1972; White et al., 1975a,b). The reason for this difference is not clear.

This dissertation describes the use of the tracer method in a series of experiments designed to examine VFA production and interconversion in the rumen of steers consuming either a whole or ground corn high-concentrate diet. The discussion presented in Chapter II outlines the general theory of the tracer method. The identification of system parameters by either the single dose or constant infusion method is discussed. In addition, a new algorithm is presented describing the propagation of the experimental errors associated with the observed state variables to the errors associated with the estimated model parameters. Presented in Chapter III is a review of the current concepts of ruminant carbohydrate digestion and metabolism with emphasis toward animal production on high-concentrate diets. Chapter IV outlines the experimental methods employed in this study. Chapter V is a presentation and discussion of the experimental observations gathered during the course of this investigation. Finally, Chapter VI is a brief summary of the experimental results and concludes with recommendations for future studies investigating VFA metabolism in steers consuming high-concentrate diets.

CHAPTER II

THEORY OF COMPARTMENTAL ANALYSIS

Introduction

Tracer kinetics is a valuable tool in the study of complex biochemical and physiological systems. Defining and structuring biochemical and physiological systems within the constraints of a compartmental system or model has yielded qualitative and quantitative information about the movement of specific atoms and molecules. A compartment model is made up of a finite number of macroscopic units, called compartments or pools, each of which is considered to be homogeneous and well mixed. These compartments interact by exchanging material. In the real world the most common type of compartmental system is an open system — one having an exchange with the environment; however, any open system can be transformed into a closed system if the inputs and outputs are simply considered as added compartments. In an open system, one or more of the compartments communicate with the environment through a series of inputs and outputs. The mathematical theory of the behavior of these systems is called compartmental analysis and, in many cases, is analogous to linear systems theory used by engineers. Engineers often can define the structural and functional relationships of their models and form input-output relationships for the device under study.

In contrast, biological scientists generally discover that experimental data describes input-output responses for a particular biochemical or physiological system which cannot be readily identified, defined or estimated. This is commonly called the inverse problem.

The theory and application of compartmental analysis is well developed. Appropriate discussions of this topic are presented in several texts and monographs as well as in the scientific literature (Sheppard, 1962; Jacquez, 1972; Rescigno and Beck, 1972; Shipley and Clark, 1972; Riggs, 1976). This review will emphasize various aspects of the tracer method so as to construct a suitable foundation to understand methods used in this thesis.

The theory and application of compartmental analysis is generally considered in three phases. The first involves the development of a plausible model describing the structure of and the existing parameters for the particular system to be studied. Modelling should be based upon biological knowledge currently available. The structure-function relationships should have meaning in terms of known biological processes and/or structure of the real system. Secondly, based upon the specific model, an analytic theory must be developed describing mathematically the relationships of the system. This is perhaps the simplest part of the modelling process. The third phase involves experimental design, estimation of parameters from experimental data and statistical analysis. The experimenter often has more than one plausible model describing the system of interest and, based on experimental data, must decide which model is "best". Design of experiments and choice of data to be collected should be, at least partly, determined by the specific model chosen. Therefore,

model selection and experimental data constantly interact. Data and theory are not independent, as is sometimes believed. This must be considered in determining which model is "best".

Definitions and Terms

For describing a compartmental system, specific terms and definitions are used. Although attempts have been made to unify tracer terminology (Brownell et al., 1968) much diversity remains in the literature. Therefore, a brief discussion of related nomenclature will follow as an aid in clarification.

Compartment

Historically, the first use of a compartment model was by Hevesey (1923), who demonstrated the uptake and loss of radioactive lead by the roots of plants. A year later, he traced the metabolism of radioactive bismuth in rabbits (Christiansen et al., 1924).

The concept of a compartment in the mathematical sense was first used by Artom et al. (1938) who developed differential equations describing the incorporation of ^{32}P into components of liver and bone. However, Sheppard (1948) was the first to use the term compartment and described it as an amount of a material which acts kinetically as a distinct, homogeneous, well-mixed substance within a biological system. Commonly, the term pool is used synonymously with compartment. However, according to Rescigno et al. (1972), a pool is any subdivision of a system, whether it can be further subdivided into a number of compartments or not. For example, in the rumen the total quantity of carbon contained in the volatile

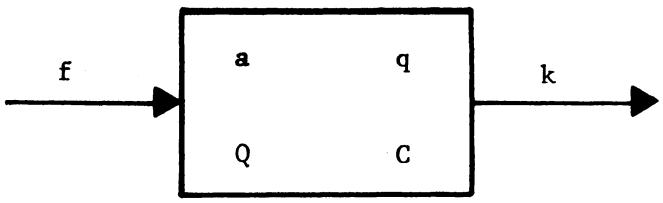
fatty acids (VFA) may be considered as a pool while the amount of carbon in extracellular acetate may be described as a compartment. Obviously, the kinetic behavior of a pool versus a compartment will differ. For purposes of this thesis, a compartment and pool will be considered as separate entities; however, according to the definition of a pool (Rescigno et al., 1972) any compartment can be considered a pool. For clarity sake, when pool is underlined (pool) it will signify the discussion refers to a sub-system containing two or more compartments.

The standard diagrammatic representation of a compartment is a box with arrows indicating flow into or out from the material storage as shown in Figure 1. Usually letters or symbols are placed inside the box which represent parameters describing concentration (C), quantity of unlabeled material (Q), amount of tracer (q) or specific activity (a). These entities are often called the state variables of the system. A basic assumption of compartmental analysis is that the quantity of unlabeled material in the various compartments remains constant over time. This is referred to as steady-state and can be defined simply as a dynamic equilibrium in the system. This constraint dictates that total input to a compartment must equal total compartmental output.

Turnover

For a system in steady-state, the total rate at which material enters or leaves a compartment is called the turnover rate. Turnover rate often is called the total flow rate, since it has the units of mass per unit time. An equally important concept is the fractional

Figure 1. A One Compartment System.


f
a
q
k
Q
C

transfer coefficient or rate constant (k in Figure 1), and is defined as that fraction of material which leaves a compartment per unit time. These terms are related by the expression,

$$f = k\,Q \tag{2.1}$$

where f represents the turnover rate or flow of material in terms of mass units per unit time, k is the rate constant associated with the loss of material from the compartment in units of $(\text{time})^{-1}$ and Q is the total amount of material in the compartment, expressed in mass units. Often times Q is referred to as the pool size, although it is actually compartment size.

In a dynamic steady-state, compartmental material is constantly being replaced with new material. The turnover time is defined as that time which is required to completely replace the quantity of material, Q, in a compartment. It is related to the fractional rate constant by

$$T = k^{-1} \tag{2.2}$$

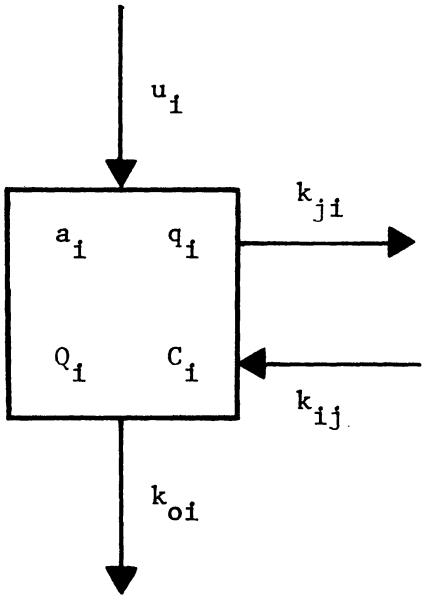
where T is the compartmental turnover time expressed in units of time.

In a complex compartmental system, as illustrated in Figure 2, these basic relationships still apply. However, turnover rate becomes the total of all the rates leaving the compartment; i.e.,

$$f_{ii} = \sum_{\substack{j=0 \\ j\neq i}}^{n} f_{ji} = \sum_{j=0}^{n\,\prime} f_{ji} \tag{2.3}$$

where Σ' indicates the summation of terms for all $j\neq i$, f_{ji} is the flow

Figure 2. General Configuration of an Open Multi-Compartmental System.


u_i
k_{ji}
a_i
q_i
Q_i
C_i
k_{ij}
k_{oi}

from compartment i to compartment j and f_{ii} represents the total turnover rate of compartment i in an n-compartment system. Therefore, in a complex system, turnover time becomes

$$T_i = k_{ii}^{-1} \tag{2.4}$$

where k_{ii} is the sum of all rate constants of exit associated with compartment i; i.e.,

$$k_{ii} = \sum_{j=0}^{n} k_{ji}. \tag{2.5}$$

Tracers

The constraint of steady-state conditions on unlabeled material forces the compartmental analyst to have a means of identifying specific atoms or molecules so that movement through the system can be followed or traced with a suitable detector. Ideally, these tracers (1) must behave chemically and physiologically exactly like the material or tracee being studied. Also, (2) the addition of tracer must be small enough so that addition will not perturb the system under observation and (3) the tracer must be uniformily distributed throughout the tracee. Tracers are not new to biological studies for Stewart (1897) used a single injection of hypertonic saline to estimate cardiac output in a dog. Other investigators have used stained feed particles, dyes and inert materials as tracers in the study of rate processes occuring in biological systems. It was not until the early 1920's with the advent of radioactive isotopes that physiologists and biochemists had available to them tracers with

the properties which, aside from slight mass differences, approached that of ideal.

Typically, the experimental unit to measure radioactivity is counts per unit time. This instrumental readout must be converted to disintegrations per unit time by accounting for apparent losses of radioactivity due to inefficiencies of detection. The standard unit of radioactivity is the Curie (Ci), which is defined as 3.7×10^{10} disintegrations per second. Generally, in biological systems where radioactive tracers are used, the amount of radioactivity present is expressed relative to the quantity of tracee containing the measured amount of radioactivity. A typical ratio that is calculated is disintegrations per minute (DPM):gram of tracee. This is known as the specific activity (a) of a substance and is quite often the units of the experimental results obtained from laboratory analysis. Often, radioactivity per unit of compartment, such as DPM/ml blood, is called specific activity. Although not truly specific activity, such relationships are valid if compartment concentration is constant.

In the present study, radioactive tracers such as 1-^{14}C-acetate, 2-^{14}C-propionate and 1-^{14}C-butyrate are used in a compartmental analysis of extracellular VFA's in the rumen. Furthermore, a non-radioactive tracer, polyethylene glycol (PEG), is used to estimate the turnover of the rumen liquid compartment. In this case, however, turnover refers to the outflow of liquid to the abomasum and does not include the flow or exchange of water through the rumen wall.

Mathematical Description of a Compartmental System

General

Compartmental systems are generally considered to be fundamentally constrained by the linear donor control concept. Under this scheme, compartmental outflows are defined to be proportional to the quantity of material in the donor compartment. As a result, the rates of change of the state variables of a system can be written as first order functions of these state variables. Therefore, the system is linear and can be described by a set of linear differential equations. It should be noted that the tracer system is linear in specific activity, even if the biological model is non-linear (Mohler, 1973). To date, this convenient property has grossly been unexplored as a tool in the study of the non-linear behavior of biological systems. Hopefully, in the future this pattern will change.

The general compartmental system is represented in Figure 2. The rate of change of the state variable x_i in an n-compartment system can be expressed as

$$\dot{x}_i = \sum_{j=1}^{n'} k_{ij} x_j + u_i - \sum_{j=1}^{n'} k_{ji} x_i - k_{oi} x_i, \qquad (2.6)$$

for all $i = 1, \ldots, n$, where $\dot{x}_i$ is the time derivative of x_i, k_{ij} is the rate coefficient for transfer from compartment j to compartment i, u_i represents input to the i-th compartment from the environment and k_{oi} is the rate constant for transfer from compartment i to the

environment. Letting

$$k_{ii} = \sum_{j=1}^{n}, \; k_{ji} + k_{oi}, \; (i = 1, \ldots, n), \tag{2.7}$$

then Equation (2.6) becomes

$$\dot{x}_i = \sum_{j=1}^{n}, \; k_{ij}\, x_j - k_{ii}\, x_i + u_i, \; (i = 1, \ldots, n). \tag{2.8}$$

The model described by Equation (2.8) has the properties of $k_{ij} \geq 0$ for $i \neq j$, $k_{ii} > 0$, and $u_i \geq 0$, from which it can be shown that $x_i(0) > 0$, $(i = 1, \ldots, n)$, implies $x_i(t) > 0$ $(i = 1, \ldots, n)$ for all $t \geq 0$ (Mulholland and Keener, 1974).

When the state variable x_i is in steady-state, $\dot{x}_i = 0$ for all time and from Equation (2.8)

$$k_{ii}\, x_i = \sum_{j=1}^{n}, \; k_{ij}\, x_j + u_i, \; (i = 1, \ldots, n). \tag{2.9}$$

Therefore, in steady-state, input equals output. Either of these concepts may be considered as a compartmental throughput; i.e., the movement of material through a compartment. Denoting throughput by z_i, Equation (2.9) becomes

$$k_{ii}\, x_i = z_i, \; (i = 1, \ldots, n), \tag{2.10}$$

which clearly illustrates how structure, represented by x_i, and function, k_{ii}, are related to material fluxes in biological systems

(Mulholland and Gowdy, 1977); that is,

$$\text{throughput} = \text{turnover} \times \text{content}. \tag{2.11}$$

The use of radioactive tracers in compartmental studies can generally be divided into two different types of experimental design. Of course, the objective in each experiment is to identify the k_{ij}'s or appropriate functions of the k_{ij}'s such as the f_{ij}'s. One method, known as the single dose method, requires that a single dose of tracer is rapidly delivered to a single compartment of tracee, resulting in a transient response of tracer in that compartment as well as other compartments in the system. The other method, known as the constant infusion method, involves a continuous input of tracer to a compartment and the system is observed until tracer is in steady-state in all compartments. These two methods of transient and steady-state analysis will be discussed in more detail later.

In the further development of this topic, matrix algebra techniques will be found useful in relieving some of the cumbersomeness encountered when handling large systems of equations (Pease, 1965; Thompson, 1969). These will be coupled with appropriate methods of linear systems analysis (Boyce and DiPrima, 1969; Wiberg, 1971).

Equation (2.8) expressed in vector-matrix notation is

$$\dot{\underline{x}} = \underline{K}\,\underline{x} + \underline{B}\,\underline{u} \tag{2.12}$$

where the coefficient matrix $\underline{K} = (k_{ij})$ is an n x n matrix of the fractional rate coefficients,

$$\underline{K} = \begin{bmatrix} -k_{11} & k_{12} & \cdots & k_{1n} \\ k_{21} & -k_{22} & \cdots & k_{2n} \\ \vdots & \vdots & & \vdots \\ k_{n1} & k_{n2} & \cdots & -k_{nn} \end{bmatrix},$$

the $\underline{x}$ vector represents the n compartmental contents in terms of the state variable x,

$$\underline{x} = \begin{bmatrix} x_1 \\ \vdots \\ x_n \end{bmatrix},$$

the input vector $\underline{u}$ describes the n compartmental inputs as

$$\underline{u} = \begin{bmatrix} u_1 \\ \vdots \\ u_n \end{bmatrix},$$

and the input connection matrix $\underline{B} = (b_{ij})$ is an n x n matrix with entry b_{ij} equal to one if input u_j enters compartment i, if not, the entry is zero. Usually in biochemical systems u_j enters only compartment j; therefore, $\underline{B}$ consists of the n-th order identity matrix if all inputs are present; i.e.,

$$\underline{I} = \begin{bmatrix} 1 & 0 & \cdots & 0 \\ 0 & 1 & \cdots & 0 \\ \vdots & \vdots & & \vdots \\ 0 & 0 & \cdots & 1 \end{bmatrix}.$$

If $\underline{B} = \underline{I}$, then (2.12) becomes

$$\dot{\underline{x}} = \underline{K}\ \underline{x} + \underline{u}. \tag{2.13}$$

Single Dose Method

In the single dose method, tracer is injected into compartment i very rapidly which simulates an impulse input function. Therefore, $\underline{u} = \underline{0}$ for all time $t > 0$, hence

$$\dot{\underline{x}} = \underline{K}\ \underline{x}. \tag{2.14}$$

The solution of (2.14) is described by

$$\underline{x}(t) = \underline{\Phi}(t)\ \underline{x}(0) \tag{2.15}$$

where $\underline{x}(t)$ is an n-th order solution vector, $\underline{x}(0)$ is an n-th order constant vector defined by the initial conditions of the system at $t = 0$ and $\underline{\Phi}(t)$ is an n x n matrix known as the state transition matrix or fundamental solution matrix. It can be shown by the method of successive approximations (Picard's Method) that

$$\underline{\Phi}(t) = e^{\underline{K}\ t}. \tag{2.16}$$

Since tracer is injected at $t = 0$ into only one compartment (k-th

compartment) per experiment, the following conditions apply:

$$\begin{aligned} x_i(0) &= 0, \text{ for } i \neq k \text{ and} \\ x_k(0) &= D_k, \text{ for } i = k, \end{aligned} \tag{2.17}$$

where D_k is the total dose of tracer in compartment k. Quite often experimental data are normalized with respect to D_k; therefore,

$$x_k(0) = 1 \tag{2.18}$$

and $x_i(t)$ becomes a normalized state variable defined as a state variable which has been adjusted to a fraction of dose basis.

Incorporating the normalized single dose conditions of (2.17) and (2.18) into Equation (2.15) yields

$$\underline{x}(t) = \underline{\Phi}^{(k)}(t) \tag{2.19}$$

where $\underline{\Phi}^{(k)}(t)$ is the k-th column vector from $\underline{\Phi}(t)$ and it represents a fundamental set of solutions for (2.14) given a tracer impulse to compartment k.

The identification of $\underline{\Phi}^{(k)}(t)$ must be derived from the time series values of the state variable, $x_i(t)$, for i = 1, ..., n. This requires taking the Laplace transform of (2.14) such that

$$s\,\underline{X}(s) - \underline{x}(0) = \underline{K}\,\underline{X}(s) \tag{2.20}$$

where s is a variable introduced as an integrating factor and $\underline{X}(s)$ is the Laplace transform of $\underline{x}(t)$. Solving (2.20) for $\underline{X}(s)$ gives

$$\underline{X}(s) = (s\,\underline{I} - \underline{K})^{-1}\underline{x}(0). \tag{2.21}$$

This requires the computation of $(s\ \underline{I} - \underline{K})^{-1}$. For systems ≤ fourth order, Cramer's rule can be used; but, for higher order systems other algorithms (Leverrier's) might be easier (Wiberg, 1971). Hence, the inverse transformation of Equation (2.21) is

$$\underline{x}(t) = \mathcal{L}^{-1}\{(s\ \underline{I} - \underline{K})^{-1}\}\ x(0). \tag{2.22}$$

Equating (2.15) and (2.22), it is apparent that

$$\underline{\Phi}(t) = \mathcal{L}^{-1}\{(s\ \underline{I} - \underline{K})^{-1}\}. \tag{2.23}$$

Again, considering a normalized single dose injection to compartment k and relationship (2.23), Equation (2.22) becomes

$$\underline{x}(t) = \underline{\Phi}^{(k)}(t) \tag{2.24}$$

such that

$$\underline{\Phi}^{(k)}(t) = \underline{H}^{(k)}\ \underline{e}^{-g\ t} \tag{2.25}$$

where the coefficient matrix can be written as

$$\underline{H}^{(k)} = \begin{bmatrix} h_{k11} & h_{k12} & \cdots & h_{k1n} \\ h_{k21} & h_{k22} & \cdots & h_{k2n} \\ \vdots & \vdots & \cdots & \vdots \\ h_{kn1} & h_{kn2} & \cdots & h_{knn} \end{bmatrix}$$

in which h_{kij} is the i-th compartmental coefficient corresponding to the j-th eigenvalue when tracer is injected into compartment k, and the exponential vector of eigenvalues given by

$$\underline{e}^{-g\,t} = \begin{bmatrix} e^{-g_1 t} \\ e^{-g_2 t} \\ \cdot \\ \cdot \\ \cdot \\ e^{-g_n t} \end{bmatrix}$$

where g_i is the i-th eigenvalue of the $\underline{K}$ matrix. Incidentally, $\underline{H}^{(k)}$ is the k-th column vector of $\underline{H}$ which corresponds to $\underline{\Phi}(t)$.

From the inverse transform of (2.21), the h_{kij} and g_i terms can be shown to be functions of the fractional rate constants appearing in the system matrix, $\underline{K}$. Experimentally, these coefficients and exponents are identified through various graphic (curve peeling) and computer techniques which rely on measuring slopes and intercepts of the compartmental time series data (Shipley and Clark, 1972). As a result, these rate coefficients, which describe the transfer of tracer (as well as tracee) between compartments, can be calculated. In order to obtain a complete solution in terms of all rate constants as well as all pool sizes, several independent tracer experiments may be necessary in which tracer is injected into a different compartment in each experiment. This would result in the complete identification of the $\underline{\Phi}(t)$ matrix.

Constant Infusion Method

The constant infusion method requires a steady-state analysis of the linear non-homogeneous system described by Equation (2.8). Typically, tracer is infused into only one compartment (k-th compartment) per experiment; therefore, the input, u_i, can be expressed

mathematically as

$$u_i = 0, \text{ for } i \neq k \text{ and}$$
$$u_k = 1, \text{ for } i = k \tag{2.26}$$

where u_k is the normalized input (infusion rate) to the k-th compartment. Again, $x_i(t)$ becomes a normalized state variable. Input is continued to compartment k until tracer in all compartments reaches a constant level. Therefore, $\dot{x}_i = 0$ and Equation (2.8) can be written as

$$0 = \sum_{j=1}^{n,} k_{ij} x_j - k_{ii} x_i + u_i \tag{2.27}$$

or, rearranging terms,

$$\sum_{j=1}^{n,} k_{ij} x_j - k_{ii} x_i = -u_i. \tag{2.28}$$

Now, a word about the units of the terms in Equation (2.28). Usually, in tracer experiments involving radioactive tracers, u_i is in terms of units of radioactivity per unit time (e.g., μCi/minute). Since k is defined to be in units of $(\text{time})^{-1}$, then x_i, the state variable, is forced to represent q_i, the total amount of radioactivity in the i-th compartment, which is in units of radioactivity (μCi). However, q_i is quite difficult to measure experimentally; instead, the steady-state specific activity, a_i, is the more easily obtained state variable. If the size of compartment i is already known, then

$$q_i = a_i Q_i. \tag{2.29}$$

Ordinarily Q_i is not known *a priori* or through independent measurements. This results in a_i being the only experimentally available state variable involving radioactivity. Therefore, transforming Equation (2.28) into an equation involving a_i gives

$$\sum_{j=1}^{n}, f_{ij} a_j - f_{ii} a_i = -u_i, \tag{2.30}$$

for all i = 1, ..., n, where f_{ij} is the flow rate of material from compartment j to compartment i.

Equation (2.30) describes a complete n compartment system which is characterized by n^2 unknowns, namely the f_{ij}'s. Infusion of tracer into one compartment, or into all compartments during one experiment, yields only n independent equations. A unique solution is obtained when a different compartment is infused in n separate experiments or when all compartments are infused in each of n independent experiments. Either of these experimental designs will give n^2 independent equations from which a unique solution can be derived. Consequently, if a complete n compartment system exists, then n independent experiments, each being described by (2.30), will be needed to identify the system. This will result in the combining of n different sub-systems, each described by

$$\sum_{j=1}^{n}, f_{ij} a_{jk} - f_{ii} a_{ik} = -u_{ik}, \tag{2.31}$$

for i = 1, ..., n and j = 1, ..., n, where a_{ik} is the specific activity for the i-th compartment during the k-th experiment and u_{ik} is the infusion rate to the i-th compartment during the k-th experiment. When tracer is infused into only one compartment per experiment, then

$$u_{ik} = 0, \text{ for } i \neq k. \tag{2.32}$$

On the other hand, if tracer is infused into all compartments simultaneously per experiment, then

$$u_{ik} \neq 0, \tag{2.33}$$

for all i = 1, ..., n and k = 1, ..., n. However, care must be taken in choosing u_{ik} from one experiment to the next in order to maintain linear independence between the u_{ik}'s and, subsequently, the a_{ik}'s. In other words, the ratios of the infusion rates in each experiment, k, must be different, such that

$$\begin{aligned} u_{11} : u_{21} : \ldots : u_{n1} \neq u_{12} : u_{22} \ldots : u_{n2} \neq \ldots \\ \neq u_{1n} : u_{2n} : \ldots : u_{nn} . \end{aligned} \tag{2.34}$$

If (2.31) describes a complete n compartment system, then

$$f_{ij} \neq 0 , \tag{2.35}$$

for all i = 1, ..., n and j = 1, ..., n, and Equation (2.31) becomes the matrix equation

$$\underline{F}\ \underline{A} = -\underline{U} \tag{2.36}$$

where the coefficient matrix is

$$\underline{F} = \begin{bmatrix} -f_{11} & f_{12} & \cdots & f_{1n} \\ f_{21} & -f_{22} & \cdots & f_{2n} \\ \vdots & \vdots & & \vdots \\ f_{n1} & f_{n2} & \cdots & -f_{nn} \end{bmatrix},$$

and the state variable matrix is

$$\underline{A} = \begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1n} \\ a_{21} & a_{22} & \cdots & a_{2n} \\ \vdots & \vdots & & \vdots \\ a_{n1} & a_{n2} & \cdots & a_{nn} \end{bmatrix},$$

and the input matrix is represented by

$$\underline{U} = \begin{bmatrix} u_{11} & u_{12} & \cdots & u_{1n} \\ u_{21} & u_{22} & \cdots & u_{2n} \\ \vdots & \vdots & & \vdots \\ u_{n1} & u_{n2} & \cdots & u_{nn} \end{bmatrix}.$$

Usually $\underline{U} = \underline{I}$, however, it may be desirable to infuse tracer into more than one compartment in order to maintain similar levels of specific activity. This should improve the precision of specific activity measurement in compartments where specific activity is normally quite low and difficult to detect.

When Equation (2.31) describes an incomplete n compartment

system, then at least one $f_{ij} = 0$, $i \neq j$, and the ordering of the f_{ij}'s and a_{ij}'s into the matrix Equation (2.36) is no longer valid. Instead, the system equation can be written as

$$\underline{A}^* \, \underline{f}^* = -\underline{u}^* \tag{2.37}$$

here the specific activity matrix becomes

$$\underline{A}^* = \begin{bmatrix} \underline{A}_1^* & \underline{0} & \cdots & \underline{0} \\ \underline{0} & \underline{A}_2^* & \cdots & \underline{0} \\ \vdots & \vdots & & \vdots \\ \underline{0} & \underline{0} & \cdots & \underline{A}_n^* \end{bmatrix}, \tag{2.38}$$

the vector of flow rates is given by

$$\underline{f}^* = \begin{bmatrix} \underline{f}_1^* \\ \underline{f}_2^* \\ \vdots \\ \underline{f}_n^* \end{bmatrix}, \tag{2.39}$$

and the input vector

$$\underline{u}^* = \begin{bmatrix} \underline{u}_1^* \\ \underline{u}_2^* \\ \vdots \\ \underline{u}_n \end{bmatrix}. \tag{2.40}$$

$\underline{A}_i^*$ is an n x m matrix ($m \leq n$) and $\underline{f}_i^*$ is a m x 1 vector derived by partitioning the system matrix such that $\underline{A}_i^*$ and $\underline{f}_i^*$ are conformable. The description of $\underline{A}_i^*$ can best be done by taking the matrix transpose of $\underline{A}$, yielding

$$\underline{A}^T = \begin{bmatrix} a_{11} & a_{21} & \cdots & a_{n1} \\ a_{12} & a_{22} & \cdots & a_{n2} \\ \vdots & \vdots & & \vdots \\ a_{1n} & a_{2n} & \cdots & a_{nn} \end{bmatrix} \tag{2.41}$$

and letting

$$\underline{f}_i^* = \begin{bmatrix} f_{i1} \\ f_{i2} \\ \vdots \\ f_{im} \end{bmatrix} . \tag{2.42}$$

If $f_{ij} = 0$ for any $j = 1, \ldots, n$, except $j \neq i$, then $m < n$ and $\underline{A}_i^*$ can be described as $\underline{A}^T$ in which the j-th column has been eliminated and the i-th column has been multiplied by (-1). However, if $f_{ij} \neq 0$ for all $j = 1, \ldots, n$, then $m = n$ and $\underline{A}_i^*$ is described by $\underline{A}^T$ in which the i-th column has been multiplied by (-1). The input vector $\underline{u}^*$ is partitioned such that

$$\underline{u}_i^* = \begin{bmatrix} u_{i1} \\ u_{i2} \\ \cdot \\ \cdot \\ \cdot \\ u_{in} \end{bmatrix} \tag{2.43}$$

where $\underline{A}_i^*$ and $\underline{u}_i^*$ are conformable. Providing that m = n, then

$$\underline{f}_i^* = - [\underline{A}_i^*]^{-1} \underline{u}_i^* \tag{2.44}$$

describes the solution for $\underline{f}_i^*$. If m < n, the sub-system is over determined and a Gaussian least squares estimate for the solution of Equation (2.37) must be obtained. This is done by multiplying, on the left, by the matrix transpose of $\underline{A}_i^*$. This yields

$$[\underline{A}_i^*]^T \underline{A}_i^* \underline{f}_i^* = - [\underline{A}_i^*]^T \underline{u}_i^* \tag{2.45}$$

which in turn gives

$$\underline{f}_i^* = - \left[[\underline{A}_i^*]^T \underline{A}_i^* \right]^{-1} [\underline{A}_i^*]^T \underline{u}_i^* \tag{2.46}$$

as a solution estimate for $\underline{f}_i^*$ by minimizing the sum of squares of the residuals (Thompson, 1969).

Propagation of Error

Measurements of state variables in biological systems are accompanied by error terms which give the experimenter some level of confidence in his ability to describe accurately and/or precisely the biological phenomenon under study. These error terms may represent

the standard deviation of an analytical laboratory procedure or the standard error of a biological variable measured on several experimental units (e.g., animals). In tracer experiments, it is necessary to propagate the errors in the observations, a_{ij}'s, to those associated with the derived system parameters, f_{ij}'s. The approach commonly employed involves the use of a covariance matrix of errors to calculate the least squares estimates of the unknown parameters (Allen, 1975). Unfortunately, this covariance matrix is generally unknown or is difficult to estimate and apply. The following algorithm provides a method for approximating the errors in the f_{ij}'s given the errors in the a_{ij}'s. This algorithm appears to be much simpler to use and should give reasonable estimates of the errors associated with the f_{ij}'s (Mulholland, 1976).

Consider the system described by (2.36) and let

$$\underline{A} = \underline{M} \pm \underline{E} \tag{2.47}$$

where M is an n x n matrix with elements m_{ij} equal to the average specific activity of compartment i during experiment j, $\bar{a}_{ij}$, i.e.,

$$m_{ij} = \bar{a}_{ij} = \frac{1}{y} \sum_{p=1}^{y} a_{ijp} \tag{2.48}$$

where y is the number of sample observations and a_{ijp} is the specific activity of the p-th observation in the i-th compartment during the j-th experiment. Furthermore, $\underline{E}$ is an n x n matrix with elements e_{ij} indicating the error term associated with $\bar{a}_{ij}$. Therefore, Equation (2.36) becomes

$$\underline{F}\ (\underline{M} \pm \underline{E}) = -\underline{U} \tag{2.49}$$

which in turn gives

$$\underline{F} = -\underline{U}\ (\underline{M} \pm \underline{E})^{-1}. \tag{2.50}$$

To evaluate $(\underline{M} \pm \underline{E})^{-1}$, first rewrite it as

$$(\underline{M} \pm \underline{E})^{-1} = \underline{M}^{-1}\ (\underline{I} \pm \underline{E}\ \underline{M}^{-1})^{-1} \tag{2.51}$$

and note that

$$(\underline{I} \pm \underline{E}\ \underline{M}^{-1})^{-1} = \underline{I} \pm \underline{E}\ \underline{M}^{-1} \pm (\underline{E}\ \underline{M}^{-1})^{2} \pm \ldots \tag{2.52}$$

forms an infinite geometric series. This infinite series will converge if the absolute value of the eigenvalues of $(\underline{E}\ \underline{M}^{-1})$ are less than one (see Appendix A). Ignoring terms higher than first order gives

$$(\underline{M} \pm \underline{E})^{-1} \doteq \underline{M}^{-1} \pm \underline{M}^{-1}\ \underline{E}\ \underline{M}^{-1} \tag{2.53}$$

and substituting Equation (2.53) into Equation (2.50) results in

$$\underline{F} \doteq -\underline{U}\ \underline{M}^{-1} \pm \underline{U}\ \underline{M}^{-1}\ \underline{E}\ \underline{M}^{-1}\ . \tag{2.54}$$

Letting

$$\underline{F}_m = -\underline{U}\ \underline{M}^{-1} \tag{2.55}$$

and

$$\underline{F}_e = \pm\underline{U}\ \underline{M}^{-1}\ \underline{E}\ \underline{M}^{-1}\ , \tag{2.56}$$

then Equation (2.54) becomes

$$\underline{F} \doteq \underline{F}_m \pm \underline{F}_e \tag{2.57}$$

where $\underline{F}_m$ is the mean value matrix $(\bar{f}_{ij})$ and $\underline{F}_e$ is the matrix of errors associated with the $\bar{f}_{ij}$'s.

Appendix A contains sample calculations illustrating the relative amount of error incurred by ignoring terms in Equation (2.52) higher than first order.

Calculation of Compartment Size

Compartment size (Q_k), usually referred to as pool size, is determined from an experiment where a known amount of tracer, D_k, is abruptly injected into compartment k. Assuming instantaneous mixing, the tracer kinetics of each compartment is described by Equation (2.52). Tracer loss in compartment k, as measured by a decline in specific activity (a_k), is given by

$$a_k(t) = h_{k1}\, e^{-g_1 t} + h_{k2}\, e^{-g_2 t} + \ldots + h_{kn}\, e^{-g_n t}. \tag{2.58}$$

The pool size of compartment k is now calculated as

$$Q_k = \frac{D_k}{\sum_{i=1}^{n} h_{ki}} \quad . \tag{2.59}$$

Often times instantaneous mixing does not occur and/or the initial phase of the specific activity curve is poorly estimated. These errors could cause the gross over or under estimation of pool size.

Calculation of pool size from constant infusion data is quite difficult, if not impossible. Usually, constant infusion experiments

are begun with an initial dose of tracer, known as the priming dose, to the compartment(s) being infused. This usually shortens the time required to reach steady-state conditions; but, it also complicates the curve describing $a_k(t)$. This curve is now a function of the priming dose as well as the forcing function, u_k.

A coupling of steady-state and transient analysis could be used to circumvent this problem as well as alleviate some of the problems associated with non-instantaneous mixing of the initial dose. This would require the performance of a series of constant infusion experiments; and, following the achievement of steady-state conditions, the infusion pumps are turned off. This is followed by the determination of the compartmental specific activities at precise time intervals over a sufficiently long time span. Data from the constant infusion experiments are used to calculate the f_{ij}'s as shown previously. The k_{ij}'s are then determined from the time series data using an appropriate identification algorithm, such as one developed by Gowdy and Mulholland (1977). Using this technique, the flow rates and the fractional rate constants are determined from data derived from the same experimental time frame. This is, indeed, significant. The calculation of pool size becomes quite elementary since

$$Q_i = \frac{f_{ii}}{k_{ii}} \, . \tag{2.60}$$

Determination of Production Rate

The production rate (P) of tracee (tracer) atoms is defined as the rate of entry of new tracee (tracer) atoms into the compartment.

This must be distinguished from the turnover rate, f_{ii}, which includes that for recycled atoms not new to the pool. Since input equals output, the production rate must be balanced by tracee (tracer) irreversibly lost from the compartment. The latter being known as the irreversible disposal rate and is synonymous with production rate. Referring to Figure 3, it can be seen that atoms in compartment i arise directly from f_{io} and indirectly via f_{jo}. Therefore, the production rate of atoms (e.g., carbon) in compartment i can be expressed as

$$P_i = f_{io} + \sum_{j=1}^{n'} f_{jo} \left[\frac{f_{ij}}{f_{jj}} \right] . \tag{2.61}$$

The production rate, sometimes referred to as entry rate, can be calculated from data derived from constant infusion experiments resulting in the relationship

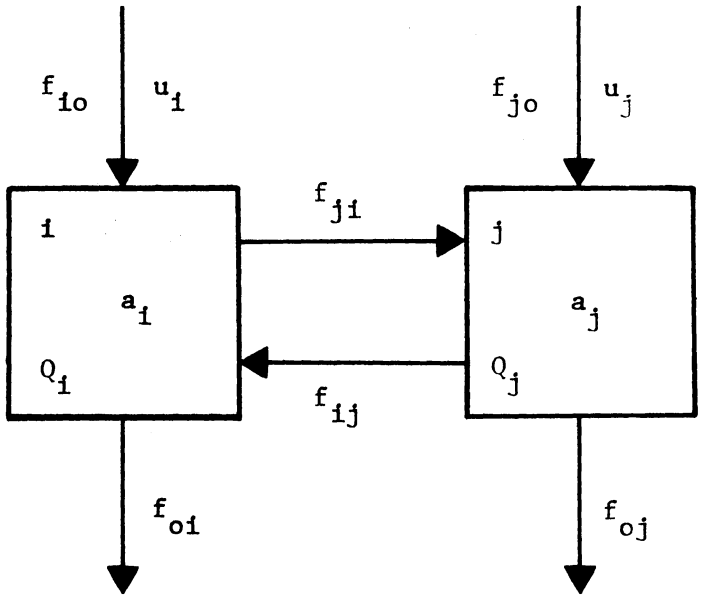
$$P_i = \frac{u_i}{a_{ie}} \tag{2.62}$$

where u_i is the infusion rate and a_{ie} is the steady-state specific activity.

When the system is in steady-state the production rate (P_i) of atoms for species i must equal the irreversal disposal rate (DR_i). Therefore, from consideration of the model in Figure 3 emerges an expression describing the irreversible disposal rate of species i,

$$DR_i = f_{oi} + \sum_{j=1}^{n'} f_{ji} \left[\frac{f_{oj}}{f_{jj}} \right] . \tag{2.63}$$

Figure 3. A General n-Compartment Model Illustrating the Relationship Between Input and Output Rates.


f_{io}
u_i
f_{jo}
u_j
f_{ji}
i
a_i
Q_i
j
a_j
Q_j
f_{ij}
f_{oi}
f_{oj}

Thus, from Equation (2.62),

$$DR_i = \frac{u_i}{a_{ie}} \cdot \tag{2.64}$$

Transfer Ratio

When studying the kinetic properties of a multi-compartment system, it is often desirable to know what fraction of the material in a particular compartment was derived from some other compartmental member of the system. Intuitively, the fraction of species j coming from species i will be equal to the net rate of material flow to j from i divided by the total net rate of material flow to (out of) j. This transfer of material from i to j is commonly termed the transfer ratio of i to j (Y_{ji}).

From the model in Figure 3, an equation for conservation of tracer in the whole system can be written as

$$u_i = f_{oi}\, a_{ie} + \sum_{j=1}^{n'} f_{oj}\, a_{je} \tag{2.65}$$

where tracer is infused only into compartment i. Divide both sides by a_{ie},

$$\frac{u_i}{a_{ie}} = f_{oi} + \sum_{j=1}^{n'} f_{oj} \left[\frac{a_{je}}{a_{ie}}\right] \tag{2.66}$$

and from Equation (2.64)

$$DR_i = f_{oi} + \sum_{j=1}^{n'} f_{oj} \left[\frac{a_{je}}{a_{ie}} \right] . \tag{2.67}$$

Thus, substituting Equation (2.63) for the left side of Equation (2.67) gives

$$f_{oi} + \sum_{j=1}^{n'} f_{ji} \left[\frac{f_{oi}}{f_{jj}} \right] = f_{oi} + \sum_{j=1}^{n'} f_{oj} \left[\frac{a_{je}}{a_{ie}} \right] \tag{2.68}$$

which can be reduced to

$$\sum_{j=1}^{n'} f_{ji} \left[\frac{f_{oj}}{f_{jj}} \right] = \sum_{j=1}^{n'} f_{oj} \left[\frac{a_{je}}{a_{ie}} \right] . \tag{2.69}$$

Rearranging Equation (2.69) yields

$$\sum_{j=1}^{n'} f_{oj} \left[\frac{f_{ji}}{f_{jj}} \right] - \sum_{j=1}^{n'} f_{oj} \left[\frac{a_{je}}{a_{ie}} \right] = 0 \tag{2.70}$$

and combining terms

$$\sum_{j=1}^{n'} f_{oj} \left[\frac{f_{ji}}{f_{jj}} - \frac{a_{je}}{a_{ie}} \right] = 0 . \tag{2.71}$$

In an unrestricted model, tracer output of compartment j must equal tracer input, i.e.,

$$a_{je} f_{jj} = f_{ji} a_{ie} + \sum_{\substack{k=1 \\ k\neq i}}^{n} f_{jk} a_{ke} . \tag{2.72}$$

If the model is restricted such that tracer input to compartment j is only derived from compartment i (infused compartment) or

$$f_{ji} >> \sum_{\substack{k=1 \\ k\neq i}}^{n} f_{jk} , \tag{2.73}$$

then

$$a_{je} f_{jj} = f_{ji} a_{ie} \tag{2.74}$$

or

$$\frac{f_{ji}}{f_{jj}} = \frac{a_{je}}{a_{ie}} . \tag{2.75}$$

By definition,

$$Y_{ji} = \frac{a_{je}}{a_{ie}} . \tag{2.76}$$

Coda

The purpose of this review has been to present current concepts of analysis of multi-compartmental systems. Many formulas have been presented during the development of this topic; however, in reality the theory is founded upon relatively few basic concepts and

assumptions. In the following pages of this manuscript some of the principles and relationships mentioned will be used in evaluating a biochemical model of VFA production in steers consuming high-concentrate rations.

CHAPTER III

LITERATURE REVIEW

Introduction

Ruminants are earth's dominant herbivores. They subsist on plant material obtained through browsing and grazing. Man, through his domestication of a few genera of ruminants (cattle, sheep and goats), has imposed widely varying planes of nutrition on these animals. Intensive agricultural production systems have been developed to meet the broad spectrum of consumer demands for products derived from today's domesticated ruminants. The diversity and intensity of these production systems is reflected in the nutritional and managerial inputs required to achieve the desired output response. In addition, the system must operate under the constraints of minimum cost and time.

An example of such a system is the feedlot phase of beef production. Diets high in readily fermentable carbohydrates, mainly starch, are fed more or less continuously to large numbers of beef cattle (Bos taurus) confined to a relatively small area of land. This situation is in sharp contrast to the one to which these animals were accustomed before entering the feedlot. In their pre-feedlot environment, the animals subsisted primarily on forage-type diets in which the carbohydrates existed largely in insoluble forms such as cellulose and hemicellulose. This remarkable degree of adaptability is mediated

through a reciprocally beneficial relationship that exists between the animal and the microbial population residing in the rumen.

Plant material ingested by the animal is fermented by the microbes to form chiefly carbon dioxide, methane and volatile fatty acids (VFAs). Through this fermentation the microbes obtain sufficient energy to maintain a relatively constant microbial mass, even though the population is undergoing continuous dilution. Concomitant with this microbial production is the absorption of the VFAs from the rumen and their subsequent oxidation by animal tissues. While VFAs provide the majority of the host's energy source, microbial cells flowing out of the rumen supply a large portion of the animals protein needs through degradation of microbial protein and absorption of amino acids in the small intestine. This symbiotic relationship between the total microbial population and the host is known as mutualism.

This review will attempt to briefly discuss various aspects of this mutualistic relationship with emphasis on the factors involved in carbohydrate utilization in ruminants consuming high-concentrate diets. For purposes of this review, a high-concentrate diet will be one containing at least 80% concentrate (grain portion plus protein supplement). The interested reader will find more extensive coverage of ruminant digestion and metabolism in Blaxter (1962), Hungate (1966) and Church (1969).

Rumen Fermentation

Overview of Rumen Function

An important aspect of ruminant digestion is the continual reduction of the particle size of the ingested feed. Diets commonly

fed to feedlot cattle vary in particle size distribution, ranging from finely ground grains to coarsely chopped hays and silages. As the food is eaten, mastication comminutes large feed particles into smaller ones, saliva is combined with the feed and the mixture is swallowed.

The composition of the saliva is 100-140 mM bicarbonate (mostly sodium bicarbonate), 10-50 mM phosphate and about 10 mM nitrogen as urea. While containing no amylolytic activity, this alkaline saliva is vital in providing proper buffering capacity in the rumen and maintaining favorable conditions for the microbial fermentation. The presence of urea signifies a unique mechanism of nitrogen conservation in the ruminant. The urea, when degraded by ruminal urease, supplies ammonium (NH_3) which is the preferred nitrogen substrate of most of the rumen microorganisms. In times of food deprivation, this mechanism of nitrogen recycling becomes increasingly important for survival of the animal.

Feed particles entering the reticulo-rumen undergo a further reduction in particle size through a process known as rumination. After the consumption of a meal, digesta in the reticulum is regurgitated into the mouth. The liquid portion is swallowed and the solids are mixed with saliva, rechewed and returned to the rumen. Rumination occurs throughout the day, interrupted by periods of resting, drinking and eating. It has been estimated that 27-39% of the time is spent ruminating (Church, 1969); however, this is quite variable and is primarily a function of the particle size of ingested feed and the level of intake (Schake, 1966). As a result, saliva production is more or less continuous, with the total daily quantity range from 1-3 times the volume of the rumen contents. The contents of the rumen

contains 10-18% (w/v) dry matter and comprises 8-15% of the total animal's weight.

Rumen contents are continuously mixed by contractions of the rumen wall which occur at 30-50 second intervals (Church, 1969). This results in a fairly homogeneous distribution of digesta in the rumen, particularly when the particle size has been reduced through mechanical processing before feeding.

Digesta flows from the rumen into the omasum. Microbes as well as undigested feed particles pass with the liquid flowing through the reticulo-omasal orifice. In the sheep this orifice acts like a high-pass filter allowing passage of particles with a mean diameter of approximately 1.5-2.0 mm or less (Hungate, 1975), while in cattle passage of larger particles is observed. Fermentation acids, bicarbonate and water are absorbed as digesta passes through the leaf-like structures of the omasum (Faichney, 1975).

Digesta flowing into the final compartment of the ruminant stomach, the abomasum, undergoes a typical gastric acid digestion prior to entering the small intestine. Intestinal activity is similar to that in most animals except that (1) sucrase activity appears to be absent (Walker, 1959), (2) digestion of starch may be limited by the low concentration of pancreatic amylase (Ørskov, 1975) and (3) an exceptionally high concentration of ribonuclease is present in pancreatic juice (Barnard, 1969).

As a result of ruminal nutrient throughput, a teeming microcosm of protozoa and bacteria develop. This growing microbial mass produces (1) fermentation acids, absorbed and oxidized by the host, and (2) microbial cells, passing from the rumen and digested in the abomasum

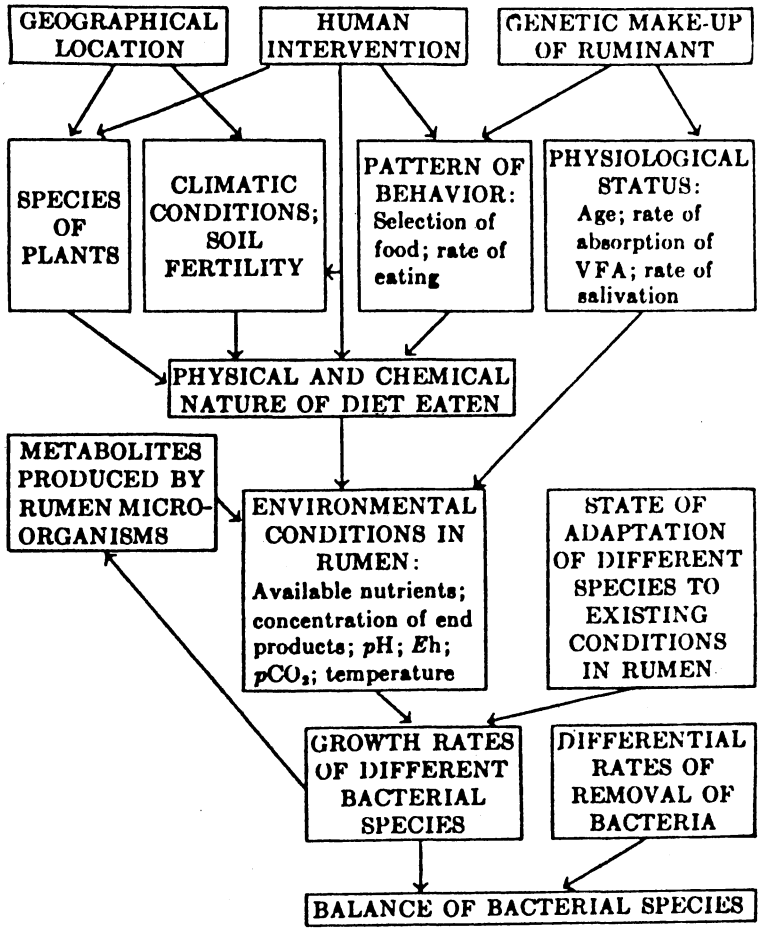
and intestine.

The contribution of the protozoa to the ruminal fermentation of high-concentrate diets is unclear. With many of the high grain diets in use today, rumen pH is often below 6.0. Such conditions usually defaunate the rumen, resulting in predominantly a bacterial fermentation with these diets. This discussion will be limited to the fermentative properties of the bacterial species predominately found in the rumen and ignore the protozoa.

The concentration of bacteria is extremely high, with approximately 10^{10}-10^{11} bacteria per milliliter of rumen fluid. Fermentation of the complex diet consumed by the ruminant is made possible by the high degree of diversity which exists in the bacterial population. The relative balance between the various bacterial species dictates to a large degree the type of fermentation which takes place; i.e., the amounts and proportions of microbial waste products produced. This balance of bacterial species is dependent on many factors as illustrated in Figure 4. Generally, this can be expressed as the principle of selection for maximum biochemical work. This simply means that those organisms accomplishing the most growth per unit time will survive or, at least, comprise a significant fraction of the population. Cell yield, limited by substrate availability, is a function of the efficiency of transforming food energy into cellular protoplasm. In the rumen the conditions and enzymes necessary to achieve maximum cell growth are too great to be possessed by any one organism. Therefore, maximum growth of the population requires a high degree of specie interdependence (Wolin, 1974).

A further constraint on the ruminal fermentation system is that

Figure 4. Factors Influencing the Balance of Bacterial Species in the Rumen. Source: Kistner (1965).


GEOGRAPHICAL LOCATION
HUMAN INTERVENTION
GENETIC MAKE-UP OF RUMINANT
SPECIES OF PLANTS
CLIMATIC CONDITIONS; SOIL FERTILITY
PATTERN OF BEHAVIOR: Selection of food; rate of eating
PHYSIOLOGICAL STATUS: Age; rate of absorption of VFA; rate of salivation
PHYSICAL AND CHEMICAL NATURE OF DIET EATEN
METABOLITES PRODUCED BY RUMEN MICRO-ORGANISMS
ENVIRONMENTAL CONDITIONS IN RUMEN: Available nutrients; concentration of end products; pH; Eh; pCO_2; temperature
STATE OF ADAPTATION OF DIFFERENT SPECIES TO EXISTING CONDITIONS IN RUMEN
GROWTH RATES OF DIFFERENT BACTERIAL SPECIES
DIFFERENTIAL RATES OF REMOVAL OF BACTERIA
BALANCE OF BACTERIAL SPECIES

it proceeds under strict anaerobic conditions. The concentration of dissolved oxygen in rumen contents has been calculated to be as low as 10^{-22} M (Hungate, 1975). This is based on the oxidation-reduction potential of -0.35 V as measured by the platinum electrode.

In an anaerobic type of fermentation, organic compounds serve as primary electron donors as well as terminal electron acceptors. As a result, this must lead to the accumulation of quite large quantities of reduced metabolites (VFAs and methane) that are the products of a reduction of the terminal electron acceptors. Compared to aerobic respiration, anaerobic fermentation is energetically less productive; i.e., fewer moles of ATP (adenosine triphosphate) per mole of glucose metabolized. Furthermore, aerobic metabolizing systems typically transform 60-70% (w/w) of the carbohydrate substrate into cellular protoplasm while in anaerobic systems this incorporation amounts to only about 10%, rarely greater than 20% (Morris, 1975). However, it is generally agreed that Y_{ATP} values are similar in aerobes and anaerobes metabolizing similar substrates at comparable growth rates.

Thus, the overall efficiency of the ruminant animal is limited by the biochemical transformations of the ruminal ecosystem (Bergen and Yokoyama, 1977). Perhaps this is the price the ruminant must pay for retaining the adaptability necessary to survive on such diverse diets.

Pathways of Volatile Fatty Acid Formation

Carbohydrate substrates entering the rumen are fermented to form microbial cells and microbial waste products. These waste products are chiefly acetic, propionic and butyric acids, carbon dioxide and methane. Also minor amounts of isobutyric, 2-methylbutyric,

isovaleric, valeric and caproic acids are produced. These waste products arise primarily from enzymatic attack on the complex polysaccharides present in the diet.

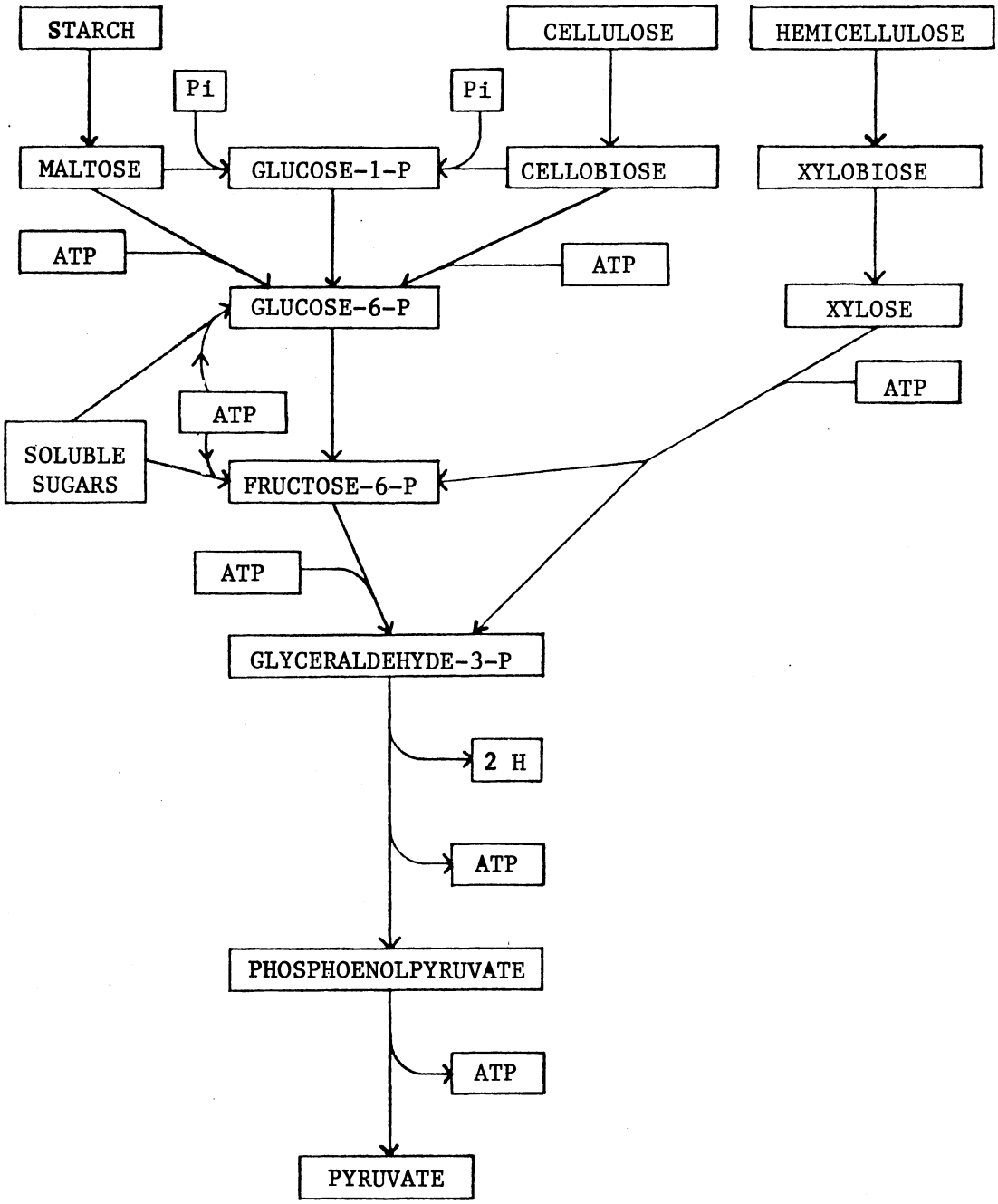
Starches, amylose (α-1,4-glucan) and amylopectin, and dextrans are the predominant forms of carbohydrate present in high-concentrate rations. Bacterial amylases (Nasr, 1950) are responsible for the cleavage of these large molecules into maltose units. Palmquist and Baldwin (1966) have demonstrated the specific activity (mg maltose produced/min/mg protein) of α-amylase to increase with increasing dietary concentrates. Detailed information concerning bacterial interactions and specific pathways involved in the degradation of these polysaccharides to their monomeric units is still lacking. However, it is possible that these maltose units are cleaved by a maltose phosphorylase (Hobson and MacPherson, 1952) catalyzing the reaction

$$\text{maltose} + \text{Pi (inorganic phosphate)} \longrightarrow \text{glucose-1-P} + \text{glucose.} \qquad (3.1)$$

Perhaps this mechanism is similar to the phosphorolytic cleavage of cellobiose by Ruminococcus flavefaciens (Ayers, 1959). Hydrolytic splitting of maltose yielding two glucose units may also exist (Thomas, 1960; Walker, 1965).

Other complex polysaccharides such as cellulose (β-1,4-glucan) and hemicellulose (β-1,4-xylan) exist in ruminant feeds. Usually, these make up only a small fraction of the total carbohydrate supply in high-concentrate diets. At any rate, these complex polysaccharides are converted to three carbon intermediates almost exclusively via the glycolytic Embden-Meyerhof pathway (Baldwin et al., 1963; Baldwin, 1965). Figure 5 represents a diagrammatic summary of the carbon flow

Figure 5. Diagrammatic Outline of the Degradation of the Major Carbohydrate Fractions Commonly Found in Ruminant Feeds.


STARCH
CELLULOSE
HEMICELLULOSE
Pi
Pi
MALTOSE
GLUCOSE-1-P
CELLOBIOSE
XYLOBIOSE
ATP
ATP
GLUCOSE-6-P
XYLOSE
ATP
ATP
SOLUBLE SUGARS
FRUCTOSE-6-P
ATP
GLYCERALDEHYDE-3-P
2 H
ATP
PHOSPHOENOLPYRUVATE
ATP
PYRUVATE

from these complex carbohydrates to pyruvate.

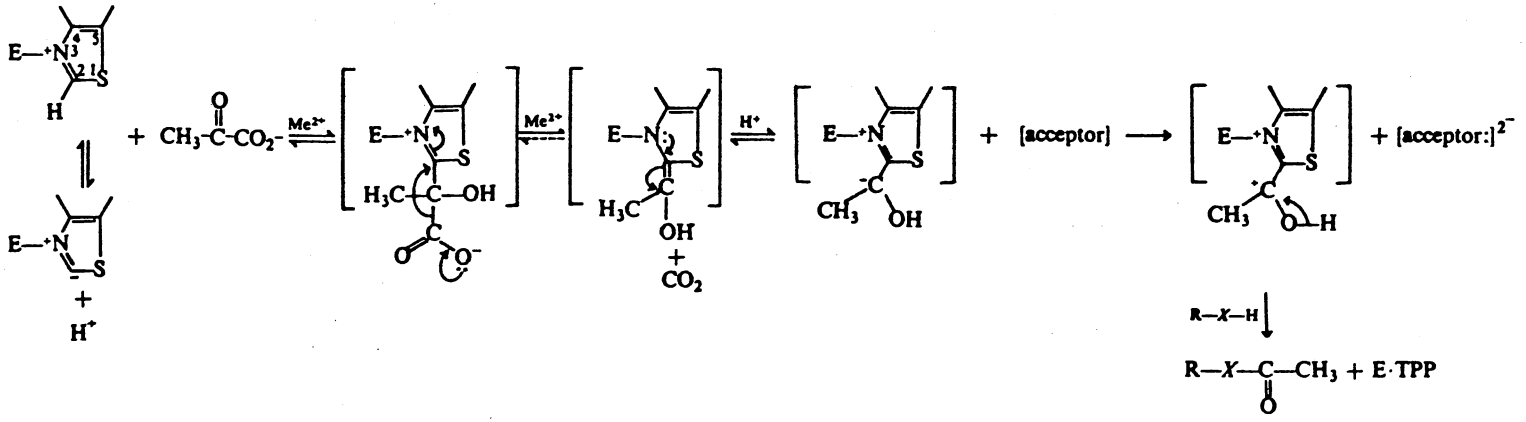
The next phase of ruminal fermentation is the conversion of pyruvate to acetate, propionate, butyrate, CO_2 and CH_4. The pathways involved in these metabolic transformations have been reviewed by Baldwin (1965), Leng (1970) and Demeyer and Van Nevel (1975). Therefore, only the salient features of each of the pathways involved shall be presented.

Acetate. The phosphoroclastic reactions appear to be the major pathways by which pyruvate is decarboxylated to acetate (Wolfe and O'Kane, 1955; McCormick et al., 1962). Two distinct pathways involving the clastic reaction (Mahler and Cordes, 1971, p. 518) are present in the anaerobic fermentation of pyruvate to acetate (Baldwin, 1965). These reactions are illustrated in Figure 6. Both systems require coenzyme A (CoASH), thiamine pyrophosphate (TPP), Fe^{++} and possibly Mg^{++}.

The clostridial phosphoroclastic (thioclastic) system is depicted in Figure 6(a) in which the $[\text{acceptor}]_{ox} = H^+$ and $[\text{acceptor}]_{red} = H_2$. The formation of H_2, coupled to the decarboxylation of pyruvate, is thought to occur via the electron-carrying protein ferredoxin (fd) (Mortenson et al., 1963). In the presence of the enzyme hydrogenase, H^+ can function as an electron acceptor leading to the formation of molecular hydrogen (Mahler and Cordes, 1971, p. 574). For the clostridial system this can be expressed as

$$\text{pyruvate} + \text{E}\bullet\text{TPP} + 2\ \text{fd}_{ox} + \text{CoASH} \longrightarrow$$
$$\text{acetyl-S-CoA} + \text{E}\bullet\text{TPP} + CO_2 + 2\ \text{fd}_{red} \qquad (3.2)$$

Figure 6. Acetate Formation via the Clastic Reaction.



Source: Mahler and Cordes (1971, p. 518).

(a) CLOSTRIDIAL PHOSPHOROCLASTIC REACTION

$Me^{2+} = Fe^{2+}$; R-X-H = CoA-S-H ; [Acceptor] = H^{+} ; $[Acceptor:]^{2-} = H_2$

(b) FORMATE PHOSPHOROCLASTIC REACTION

$Me^{2+} = Mn^{2+}$; R-X-H = CoA-S-H ; [Acceptor] = CO_2 ; $[Acceptor:]^{2-}$ = Formate

$$2\ fd_{red} + 2\ H^{+} \longrightarrow 2\ fd_{ox} + H_2 \qquad (3.3)$$

where E•TPP is the enzyme-cofactor complex, fd_{ox} is oxidized fd and fd_{red} is reduced fd. Reaction (3.2) is catalyzed by pyruvate decarboxylase (EC 4.1.1.1) while reaction (3.3) requires the enzymes transhydrogenase and hydrogenase.

Figure 6(b) illustrates the formate phosphoroclastic system in which CO_2 = $[acceptor]_{ox}$ and formate = $[acceptor]_{red}$. The end-products from this reaction, H_2 and formate, do not generally accumulate in the rumen and are believed important intermediates in the formation of methane (Demeyer and Van Nevel, 1975).

The net result of the clastic reaction can be written as

$$E\bullet TPP + pyruvate + [acceptor]_{ox} + CoASH \xrightarrow{Fe^{++},\ Mg^{++}} acetyl\text{-}S\text{-}CoA + [acceptor]_{red} + E\bullet TPP. \qquad (3.4)$$

Acetyl-S-CoA may participate metabolically as the active form of acetate or it may be converted to acetate via acetyl phosphate (acetyl-P) as illustrated in the following reactions:

$$acetyl\text{-}S\text{-}CoA + Pi \longrightarrow acetyl\text{-}P + CoASH \qquad (3.5)$$

$$acetyl\text{-}P + ADP \longrightarrow acetate + ATP \qquad (3.6)$$

Reaction (3.5) is catalyzed by phosphotransacetylase (EC 2.3.1.8) while acetate kinase (EC 2.7.2.1) catalyzes reaction (3.6). Therefore, each molecule of pyruvate undergoing the clastic reaction to formate, or CO_2 plus H_2 is capable of yielding one additional molecule of ATP. The conversion of pyruvate to acetate and CO_2 represents one of the primary

pathways of energy metabolism in rumen microorganisms (Baldwin, 1965).

Acetate may also arise from the β-oxidation of butyrate and longer chain fatty acids. Although this conversion may be an important energy yielding process in bacteria possessing electron transport-linked phosphorylation, the extent of its contribution in the rumen is unknown (Leng, 1970).

The reversal of reactions (3.2) and (3.3) is known to occur in Clostridium pasteurianum (Bachofen et al., 1964). Recent evidence (Sauer et al., 1975) suggests that the reductive carboxylation of acetate to pyruvate is a fundamental reaction of primary importance in rumen microorganisms. The mechanism involved is unknown and is probably not a simple reversal of reactions (3.2) and (3.3); however, ferredoxin is known to be required. This reaction, catalyzed by pyruvate synthase, is

$$\text{acetyl-S-CoA} + CO_2 + fd_{red} \longrightarrow \text{pyruvate} + \text{CoASH} + fd_{ox}. \qquad (3.7)$$

The evidence is primarily based on the fact that amino acids derived directly from pyruvate can become specifically labeled from labeled acetate (Emmanuel et al., 1974; Sauer et al., 1975).

Propionate. Two pathways are known for the conversion of pyruvate to propionate. The first one, shown in Figure 7, involves the formation of succinate and is known as the dicarboxylic acid or randomizing pathway. The second pathway, known as the direct reductive pathway, involves the formation of acrylate and is illustrated in Figure 8. The two pathways have been identified by observing the distribution of ^{14}C in propionate formed from position-labeled sub-

Figure 7. Diagrammatic Sketch of the Dicarboxylic Acid Pathway Involved in Propionate Synthesis in the Rumen. The numbers in the figure correspond to the following enzymes: (1) phosphoenolpyruvate carboxytransphosphorylase, (2) pyruvate kinase, (3) pyruvate carboxylase, (4) malic enzyme, (5) malate dehydrogenase, (6) fumarase, (7) succinate dehydrogenase, (8) succinyl-CoA transferase, (9) methylmalonyl-CoA mutase, (10) methylmalonyl-CoA racemase, (11) propionyl-CoA carboxylase, (12) transcarboxylase.

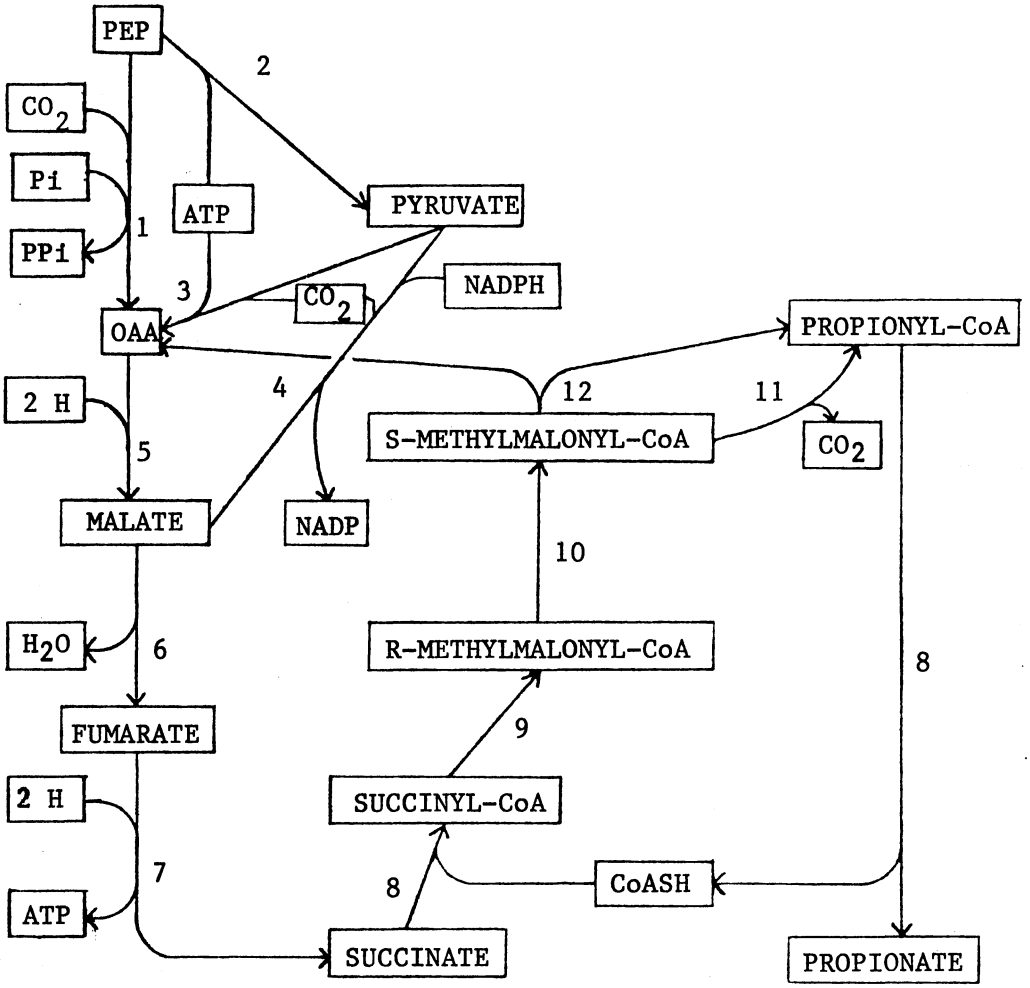
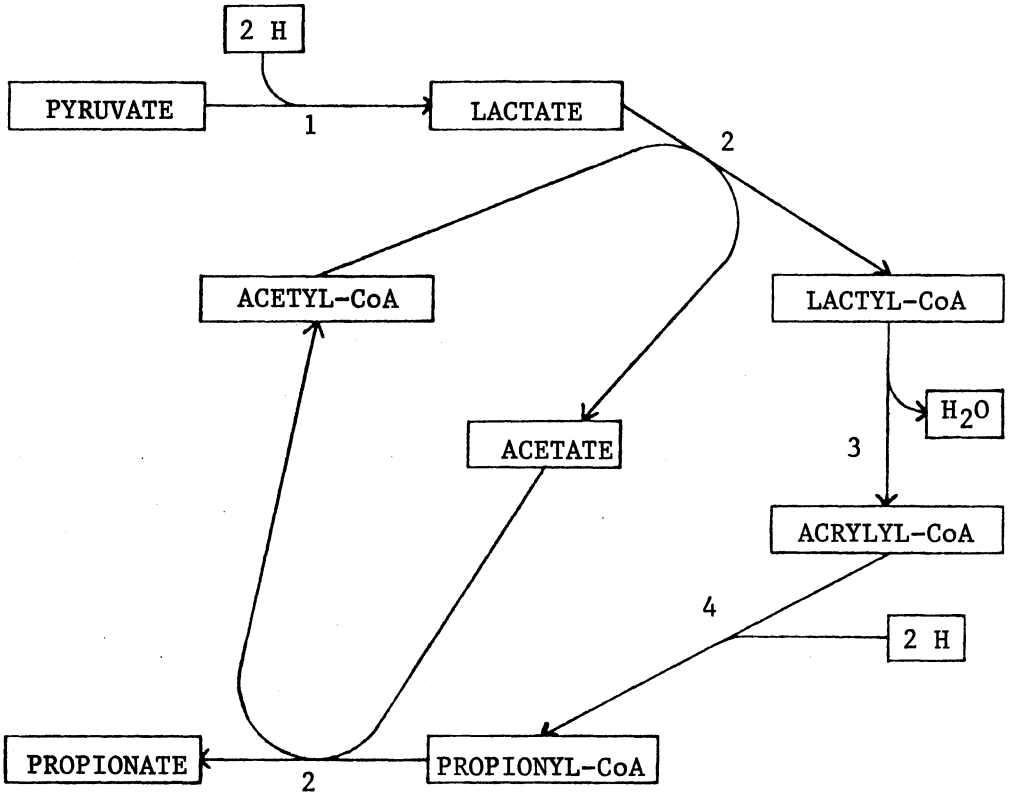

PEP
2
CO_2
Pi
ATP
PYRUVATE
1
PPi
3
CO_2
NADPH
OAA
PROPIONYL-CoA
2 H
4
12
11
5
S-METHYLMALONYL-CoA
CO_2
MALATE
NADP
10
H_2O
6
R-METHYLMALONYL-CoA
8
FUMARATE
9
2 H
SUCCINYL-CoA
7
8
CoASH
ATP
SUCCINATE
PROPIONATE

Figure 8. Diagrammatic Sketch of the Direct Reductive Pathway Involving Propionate Formation. The numbers on the figure correspond to the following enzymes: (1) lactate dehydrogenase, (2) acetic thiophorase, (3) β-hydroxyacyl-CoA hydro-lyase, (4) acrylyl-CoA reductase.


2 H
PYRUVATE
1
LACTATE
2
ACETYL-CoA
LACTYL-CoA
H_2O
ACETATE
3
ACRYLYL-CoA
4
2 H
PROPIONATE
2
PROPIONYL-CoA

strates (Baldwin et al., 1962; Baldwin et al., 1963). The relative flux of carbon through each pathway appears to be dependent upon the type of diet. Increasing the proportion of concentrate in the diet shunts relatively more carbon through the direct reductive pathway, presumably reflecting an increase in the number of microorganisms which possess the enzymes of the acrylate pathway (Wallnöfer et al., 1966; Baldwin et al., 1963).

The dicarboxylic acid pathway is a complex set of enzymatic reactions in which pyruvate or phosphoenolpyruvate (PEP) carbon is converted to propionate by several different routes. No single bacterial specie possesses all the possible routes. Generally, the pathway involves the condensation of PEP or pyruvate with CO_2 to form oxalacetate or malate according to the scheme given in Figure 7. Malate is dehydrated forming fumarate which, in turn, is reduced to succinate. Succinate is then activated by succinyl-CoA transferase which catalyzes the transfer of CoASH from propionyl-S-CoA to succinate. Succinyl-S-CoA is isomerized to form methylmalonyl-S-CoA and decarboxylation of methylmalonyl-S-CoA forms propionyl-S-CoA.

Although ATP is not known to be produced by this pathway, Hobson and Summers (1972) have proposed as many as 3 ATP per mole of propionate formed from pyruvate. These researchers suggest that such high ATP yields are necessary to explain the high growth yields of some rumen bacteria producing large amounts of propionate (Bauchop and Elsden, 1960). Some experimental evidence suggests that formation of succinate and propionate via the randomizing pathway generates ATP through (1) phosphorylation during electron transport, involving cytochrome-b and flavoproteins (de Vries et al., 1974), (2) malic

enzyme reduction (Joyner and Baldwin, 1966), and (3) methylmalonyl-S-CoA decarboxylation (Gunsalus and Schuster, 1961).

In the conversion of pyruvate to propionate via succinate, sufficient free energy is released to account for ATP production at each of the previously mentioned steps (Hobson and Summers, 1967); however, proof that this energy is captured in the form of ATP is lacking. Generally, however, one ATP is assumed to be produced in the synthesis of propionate from pyruvate (Walker, 1965), presumably occuring during anaerobic electron transport to fumarate (de Vries et al., 1974).

The conversion of pyruvate to propionate via the direct reductive pathway involves the conversion of pyruvate to lactate. As shown in Figure 8, this is followed by the activation of lactate to lactyl-S-CoA by a lactyl-S-CoA transferase, presumably requiring catalytic amounts of acetate in which the CoASH is transfered from propionyl-S-CoA to acetate. Lactyl-S-CoA is dehydrated to give acrylyl-S-CoA which, in turn, is reduced to propionyl-S-CoA. The CoASH is transfered from propionyl-S-CoA yielding propionate.

The synthesis of lactate from propionate is also known to occur by the reversal of this pathway (Mahler and Cordes, 1971, p. 600); however, the importance of this reaction in rumen bacteria is unknown and is assumed to be negligible.

The reduction of lactate to propionate provides a sink for excess reducing equivalents; however, this reaction has been considered to have no ATP yielding steps. The recent discovery of a phospholactyl-S-CoA intermediate in propionate production suggests the possibility of electron transport-linked phosphorylation (Anderson

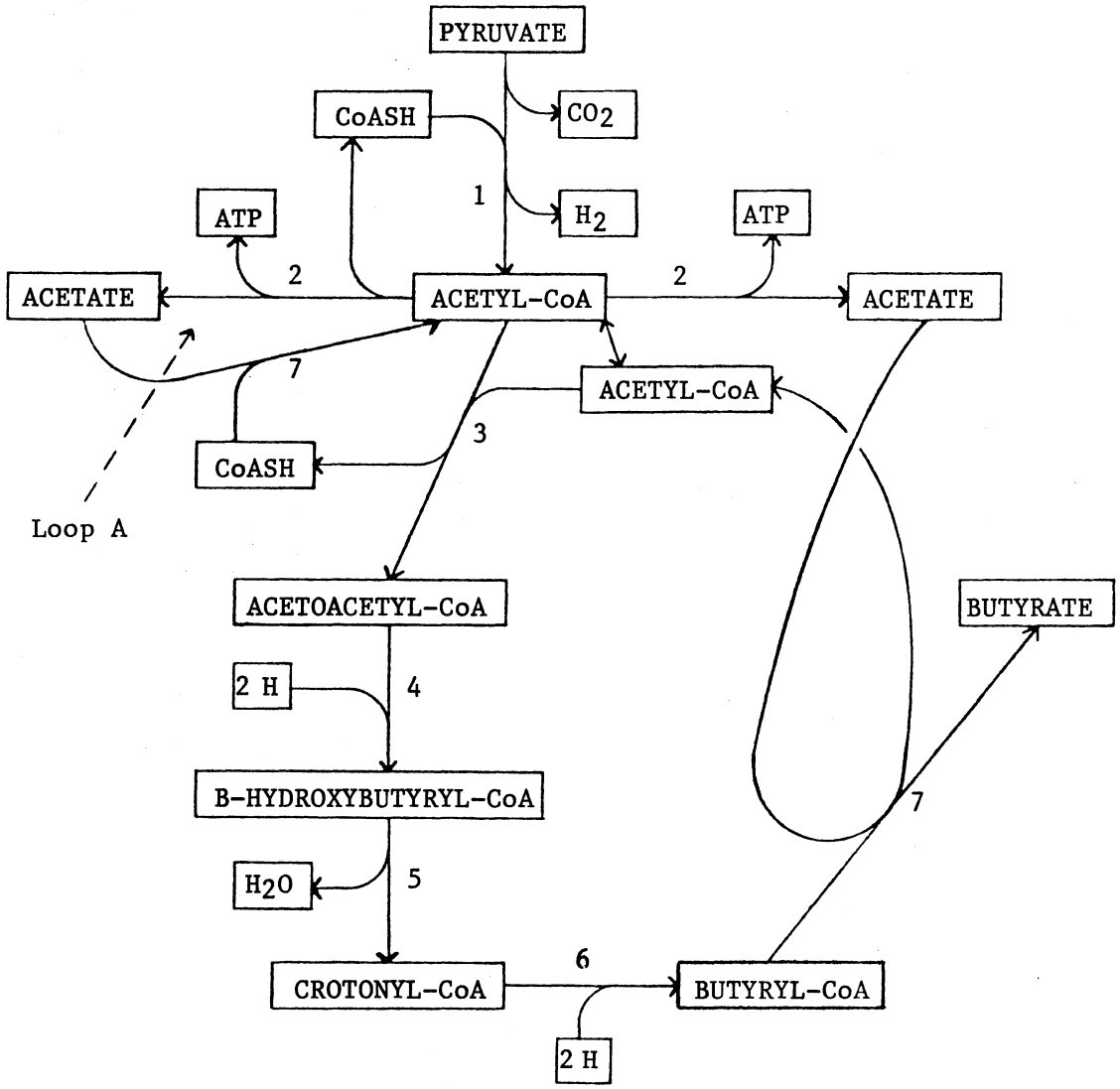
and Wood, 1969). Swick (1962) reports the isolation of a pyridine nucleotide-linked acrylyl-S-CoA reductase in Propionibacterium shermanii; however, it is unknown whether this is coupled to electron transport dependent phosphorylation.

Butyrate. Two pathways are possible for the synthesis of butyrate in anaerobic rumen microorganisms. One involves the familiar malonyl-CoA pathway while the other appears to be a reversal of β-oxidation (Baldwin, 1965). Goldman et al. (1962) have shown that in Clostridium kluyveri butyrate is synthesized by reversal of β-oxidation but that higher fatty acids were synthesized by the malonyl-CoA pathway. The relative quantitative importance of these two pathways in the rumen is unknown, but the most likely pathway involved in butyrate synthesis appears to be through the reversal of β-oxidation (Leng, 1970).

An outline of the pathway involved in butyrate synthesis is given in Figure 9. The first step, catalyzed by acetoacetyl-CoA thiolase, involves the formation of acetoacetyl-CoA from the condensation of two acetyl-S-CoA molecules. Acetoacetyl-CoA is hydrogenated through the action of β-hydroxybutyryl-CoA dehydrogenase forming β-hydroxybutyryl-CoA. Dehydration by enoyl hydrase results in the formation of crotonyl-CoA. Crotonyl-CoA is subsequently reduced by a fatty acyl-CoA dehydrogenase forming butyryl-CoA. The terminal step in butyrate synthesis, catalyzed by acetic thiophorase, involves the transfer of the CoASH moiety from butyryl-CoA to acetate, generating acetyl-CoA. This acetyl-CoA can now be used in the synthesis of another molecule of butyrate as previously outlined.

The mechanism through which electrons are transfered to β-hydroxy-

Figure 9. Diagrammatic Outline of Butyrate Synthesis via Reversal of β-Oxidation. The numbers in the figure correspond to the following reactions or enzymes: (1) Reactions (3.2) and (3.3), (2) Reactions (3.6) and (3.7), (3) acetoacetyl-CoA thiolase, (4) β-hydroxybutyryl-CoA dehydrogenase, (5) enoyl hydrase, (6) acyl-CoA dehydrogenase, (7) acetic thiophorase.


PYRUVATE
CoASH
CO_2
1
H_2
ATP
ATP
2
2
ACETATE
ACETYL-CoA
ACETATE
7
ACETYL-CoA
3
CoASH
Loop A
ACETOACETYL-CoA
BUTYRATE
2 H
4
B-HYDROXYBUTYRYL-CoA
7
H_2O
5
6
CROTONYL-CoA
BUTYRYL-CoA
2 H

butyryl-CoA and crotonyl-CoA in anaerobic bacteria is unknown; however, Baldwin and Milligan (1964) have shown that NADH (reduced nicotinamide adenine dinucleotide) appears to be the electron donor in the acyl-CoA dehydrogenase reaction. It has been postulated that electron transport-linked phosphorylation may be coupled to the acyl-CoA dehydrogenase reaction (Baldwin and Milligan, 1964), but experimental evidence is lacking.

Butyrate synthesis may function as an important electron sink within some microorganisms, utilizing reducing equivalents produced during the formation of acetate and/or pyruvate. The following sequence of reactions may describe butyrate synthesis in organisms metabolizing pyruvate via reactions (3.2) and (3.3):

$$2\ Pyr \longrightarrow Ac\text{-}S\text{-}CoA + Ac + ATP + 2\ CO_2 + 2\ H_2 \qquad (3.8)$$

$$2\ Ac\text{-}S\text{-}CoA + 4\ H^+ \longrightarrow CoASH + Bu\text{-}S\text{-}CoA \qquad (3.9)$$

$$Bu\text{-}S\text{-}CoA + Ac \longrightarrow Ac\text{-}S\text{-}CoA + Bu \qquad (3.10)$$

The net result being

$$2\ Pyr + 4\ H^+ \longrightarrow Bu + ATP + 2\ CO_2 + 2\ H_2. \qquad (3.11)$$

Thus, an organism synthesizing butyrate would gain one less molecule of ATP per two molecules of pyruvate than an organism catabolizing pyruvate to only acetate. However, this may not be true if electron transport-linked phosphorylation is coupled to these reactions.

Alternately, if two molecules of pyruvate are both converted to acetate and subsequently reactivated to acetyl-CoA by acetic thiophorase (loop A, Figure 9); then two molecules of ATP could be derived from the catabolism of two molecules of pyruvate during the synthesis

of one molecule of butyrate. Of course, this would require two molecules of acetyl-CoA to be initially present.

Branched and Straight Chain (C > 4) Volatile Fatty Acids. The presence of branched and straight chain (C > 4) volatile fatty acids in rumen fluid is common. These volatile fatty acids (isobutyric, 2-methylbutyric, isovaleric, valeric and caproic) were initially observed by el-Shazly (1952) and Annison (1954). Bentley and colleagues (Bentley et al., 1954; Bentley et al., 1955) and Bryant and Doetsch (1955) have shown these volatile fatty acids to be important growth factors for rumen microorganisms, in particular the cellulolytic bacteria. These VFAs, produced by some rumen microbes, exist as important intermediates in the catabolism (Dehority et al., 1958; Massey et al., 1976) and biosynthesis (Sauer et al., 1975) of certain amino acids. Pathways for valine, leucine, isoleucine and proline catabolism have been shown to involve isobutyrate, isovalerate, 2-methylbutyrate and valerate, respectively. Until recently, the occurence of these VFAs in the rumen was thought to be almost exclusively derived from the degradation of these amino acids. Sauer et al. (1975) presented evidence that the rumen microflora synthesizes a significant amount of amino acids de novo and that several amino acid precursors are synthesized through the reductive carboxylation of carboxylic acids normally present in the rumen. It is not known what proportion of these VFAs arise directly from these synthetic routes, or whether they arise from the de novo synthesis and subsequent degradation of the respective amino acid.

Sauer et al. (1975), using continuous cultures of mixed rumen

microorganisms, have shown specific labeling patterns of isoleucine from 1-^{14}C-propionate or 1-^{14}C-acetate and the specific labeling of leucine and valine from 1-^{14}C-acetate.

The de novo synthesis of valerate and caproate is unclear, but it may involve the condensation of acetate and propionate or three molecules of acetate, respectively. Whether this occurs via reversal of β-oxidation or the malonyl-CoA pathway is unknown.

If an organism is synthesizing valerate via reversal of β-oxidation, as was shown for butyrate, then a theoretical yield of 4 ATP/mole of hexose fermented could be calculated. A plausible pathway is illustrated in Figure 10 in which only catalytic amounts of acetyl-CoA and propionyl-CoA are required.

Methane Formation

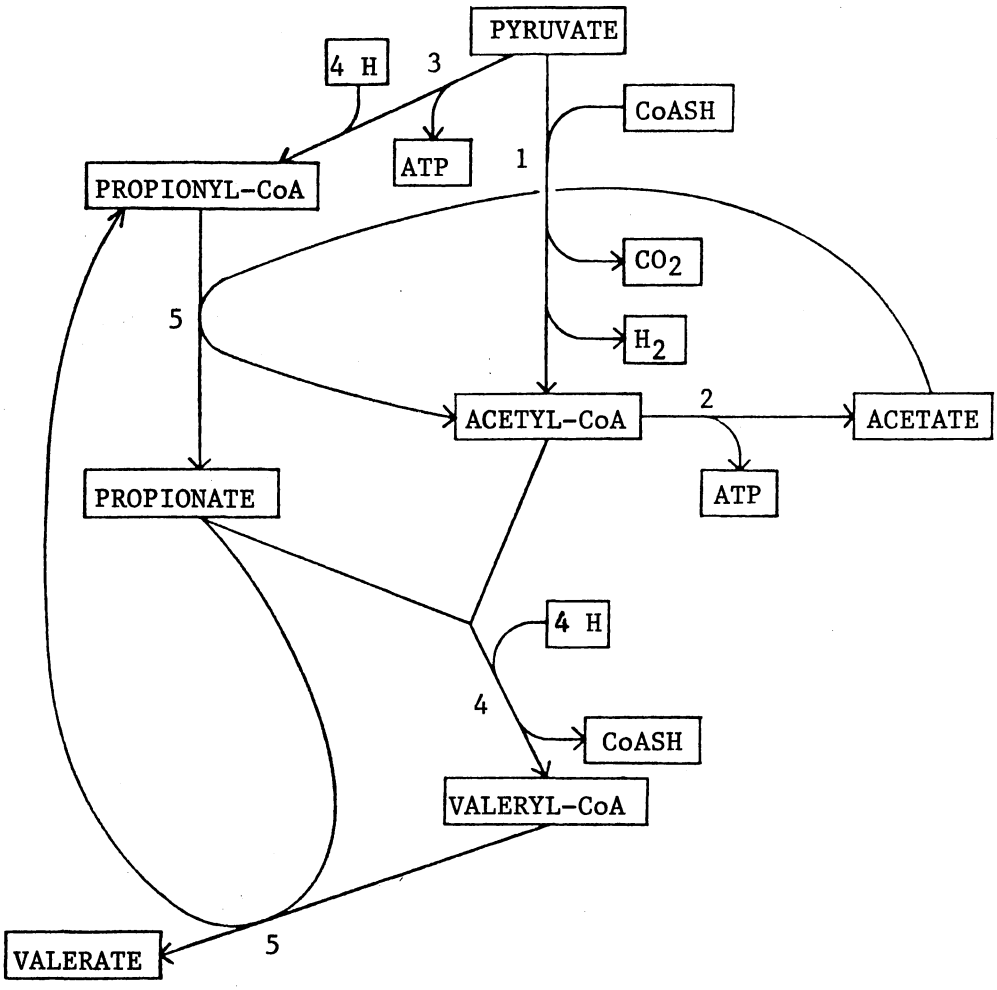
The biochemistry of methanogenesis and its significance in ruminants has been excellently reviewed by Czerkawski (1969), Wolfe (1971) and Demeyer and Van Nevel (1975). The formation of methane functions as a terminal step in the food degradation chain in the rumen. It has been proposed by Hungate (1963) that methane formation functions as an energy sink into which the excess hydrogen from all the rumen organisms drains, allowing them a higher yield of high energy phosphate (ATP) as well as generating additional ATP.

The net reaction for methane formation is

$$CO_2 + 4\ H_2 \longrightarrow CH_4 + 2\ H_2O \qquad (3.12)$$

and involves the coenzymes methylcobalamin (Wolin et al., 1963) and N^5-methyl tetrahydrofolate (Wood and Wolfe, 1966a). ATP is required

Figure 10. Schematic Diagram of a Possible Pathway for Valerate Synthesis. The numbers in the figure correspond to the following reactions or enzymes: (1) Reactions (3.2) and (3.3), (2) Reactions (3.6) and (3.7), (3) reactions in the dicarboxylic acid pathway, (4) reactions involved in reversal of β-oxidation, (5) acetic thiophorase.


PYRUVATE
4 H
3
ATP
CoASH
1
PROPIONYL-CoA
CO_2
H_2
5
ACETYL-CoA
2
ACETATE
ATP
PROPIONATE
4 H
4
CoASH
VALERYL-CoA
5
VALERATE

(Wolin et al., 1963; Wood and Wolfe, 1966b); however, the specific reactions involved remain obscure. The reaction is highly exergonic, -134 kJ/mole of methane formed (Demeyer and Van Nevel, 1975), suggesting the possibility of ATP production. This may involve the interconversion of folate derivatives and pyrmidine nucleotides by ferredoxin-linked hydrogenases (Benemann and Valentine, 1971); however attempts to demonstrate this in rumen bacteria have failed. In light of the evidence available it is generally assumed that the formation of one mole of methane results in the generation of one mole of ATP (Isaccson et al., 1975).

Substrates for methane synthesis (i.e., CO_2 and H_2) are formed during hexose dissimilation to acetate. Formate, generated in the formate phosphoroclastic reaction (3.4), is converted to $CO_2 + H_2$ by either the hydrogenlyase system (Gest, 1954) or the dehydrogenase, ferredoxin:NAD system (Brill et al., 1964). Hydrogen may also be formed in the reaction

$$NADH + H^+ \longrightarrow NAD^+ + H_2 \qquad (3.13)$$

(Wolin, 1975); since, the partial pressure of H_2 in the rumen, approximately 3×10^{-4} atmospheres (Hungate, 1967), makes this reaction thermodynamically favorable.

Maximum theoretical amounts of CH_4 produced in the rumen can be calculated from the molar proportions of VFAs synthesized. If a,p,b, and v are the molar proportions of hexose converted to acetate, propionate, butyrate and valerate respectively; then

$$a + p + b + v = 1 \qquad (3.14)$$

and, from Table I, the total hydrogen (2 H) formed is

$$TH = 4\ a - 2\ p + 2\ b - v \tag{3.15}$$

where TH is the total number moles of hydrogen (2 H equivalents) formed during the catabolism of one mole of hexose. Equation (3.14) into equation (3.15) gives

$$TH = 5\ a - p + 3\ b - 1 \tag{3.16}$$

and from reaction (3.12) the maximum limit of methane formation is

$$M = \frac{5\ a - p + 3\ b - 1}{4} . \tag{3.17}$$

Methane formation *in vivo* is always lower than the theoretical maximum (Ørskov, 1975). This discrepancy suggests that a portion of the hydrogen produced in hexose catabolism is used in the synthesis of cell material (Demeyer *et al.*, 1972). The magnitude of this proportion can be estimated by comparing the maximum value of methane production with values actually measured (Czerkawski, 1969). Demeyer and Van Nevel (1975) have shown recently that this discrepancy may be due to an accumulation of a reduced precursor of methane and conclude that the amount of hydrogen used in cell synthesis may be overestimated.

Summary of ATP Yield During Hexose Catabolism

The results so far discussed concerning the pathways involved in the conversion of hexose carbon to VFA carbon are summarized in Table I. From the data presented in Table I and assuming that one mole of

TABLE I

POSSIBLE QUANTITIES OF PRODUCTS ARISING DURING DEGRADATION OF HEXOSE IN THE RUMEN AND ESTIMATES OF CORRESPONDING ATP YIELD (VALUES IN MOLES)

Acid Formed	Pathway	Quantity Hexose Degraded	Quantity of Acid Produced	Net 2 H Formed	CO_2 Formed	ATP* Yield
Acetate	Clastic	a	2a	4a	2a	4a
Propionate	Randomizing	p	2p	-2p	0	4p
Propionate	Acrylate	p	2p	-2p	0	2p
Butyrate	Reverse β-oxidation	b	b	2b	2b	2b to 4b
Valerate	Reverse β-oxidation	v	v	-v	v	2v to 4v

*Increase all values by 1 ATP if substrate is hexose-P.

ATP is generated per mole of methane formed, a range can be calculated for the amount of ATP produced during the catabolism of hexose to VFAs, CO_2 and methane.

If θ represents the proportion of H_2 used in cell synthesis, then $(1-\theta)$ represents the proportion of H_2 available for methane synthesis. Often this latter value is calculated from the ratio of observed methane production to the theoretical amount of methane that could be obtained according to the proportions of VFAs produced. Therefore, the quantity of ATP generated from methane formation can be expressed as $M(1-\theta)$. Hence, from one mole of hexose

$$ATP_{min} = 4a + 2p + 2b + 2v + M(1-\theta) \tag{3.18}$$

and

$$ATP_{max} = 4a + 4p + 4b + 4v + M(1-\theta). \tag{3.19a}$$

From Equation (3.14), this simplifies to

$$ATP_{max} = 4 + M(1-\theta). \tag{3.19b}$$

If the initial substrate is hexose-P, then Equation (3.18) and (3.19b) become

$$ATP_{min} = 5a + 3p + 3b + 3v + M(1-\theta) \tag{3.20}$$

and

$$ATP_{max} = 5 + M(1-\theta). \tag{3.21}$$

In high-concentration diets, most of the fermentable substrate

is in the form of starch. Assuming starch degradation yields equal molar proportions of hexose and hexose-P, then from the considerations presented above

$$ATP_{min} = 4.5a + 2.5p + 2.5b + 1.5v + M(1-\theta) \quad (3.22)$$

and

$$ATP_{max} = 4.5 + M(1-\theta). \quad (3.23)$$

These calculations are comparable to those obtained from experiments studying ATP yield in continuous cultures of rumen bacteria. Hobson (1965) and Hungate (1963b) report that cell yields of rumen bacteria growing in continuous culture suggest that between 4 and 5 moles of ATP are synthesized per mole of hexose fermented.

Significance of Lactate

The importance of lactate as an intermediate in the formation of rumen VFA has been shown to be diet dependent (Jayasuriya and Hungate, 1959; Baldwin et al., 1962; Wallnöfer et al., 1966; Satter and Esdale, 1968). In hay-fed animals lactate functions as an intermediate for less than 8% of the total amount of substrate fermented. This value increases to approximately 17% when more readily available carbohydrates are included in the diet; i.e., increasing the proportion of grain. Variation due to diet has been partly attributed to an increase in numbers of several specie of lactate producing and lactate utilizing bacteria. However, large amounts of lactate in the diet (e.g., some silages contain 3-8% of

the dry matter as lactate) has resulted in the modification of the VFA fermentation pattern without a concomminant alteration in the microbial population (Satter et al., 1967).

Lactate catabolism primarily results in the formation of acetate (Jayasuriya and Hungate, 1959; Baldwin et al., 1962) or propionate (Wallnöfer et al., 1966). Oxidation of lactate to pyruvate followed by degradation via the clastic reaction yields acetate. In contrast, propionate is synthesized from lactate by the acrylate and the randomizing pathways (Wallnöfer et al., 1966). The relative contribution of these pathways in lactate metabolism may be largely determined by the type and number of lactate utilizers present in the rumen. The proportion of lactate converted to propionate increases as the availability of carbohydrate increases (Baldwin et al., 1962; Satter and Esdale, 1968). This increase is usually associated with an increase in the contribution of the acrylate pathway to lactate fermentation (Wallnöfer et al., 1966). The existence of the acrylate pathway has been reported in only one rumen microorganism, Megasphaera elsdenii (Baldwin et al., 1965) and appears to be the most prominent of the lactic acid-utilizing species when concentrates are fed.

The presence of acetate appears to be necessary for maximum utilization of lactate (Satter and Esdale, 1968). Extracellular acetate apparently serves as an electron acceptor and, through the formation of butyrate, provides an electron sink for the pair of hydrogen atoms released during the oxidation of lactate to pyruvate. Lactate enhances the incorporation of acetate into butyrate while reducing the movement of butyrate carbon to acetate (Davis, 1967; Bhat and Barker, 1947).

The unimportance of lactate in the normal rumen fermentation can be rationalized by considering the biochemical significance of the anaerobic production of ATP during the conversion of pyruvate to acetate. With lactate as the terminal acceptor, this important ATP generating step is lost since the hydrogen produced during glycolysis is stoichiometrically recovered in lactate. On the other hand, with methanogenesis functioning as an electron sink lactate is no longer needed for this purpose and the organism is able to generate an additional 2 molecules of ATP per hexose fermented. Hence, a doubling in the cell yield is realized.

Perhaps the primary function of the lactate utilizing bacteria is to provide stability to the rumen fermentation system during the fermentation of feeds high in readily fermentable carbohydrate. The maintenance of this stability becomes increasingly paramount during the period of adaption to these low roughage diets (Schwartz and Gilchrist, 1975).

This concludes the discussion regarding the various biochemical pathways involved in volatile fatty acid formation in the rumen. Since VFAs are microbial waste products, it has been assumed that, once excreted, they are not metabolized by the bacterial population. Satter and Esdale (1968), Emmanuel et al. (1974) and Sauer et al. (1975) have demonstrated the uptake and metabolism of extracellular VFAs by rumen bacteria. Presumably, interconversions of VFAs in the rumen, as measured by isotopic techniques, will reflect this phenomenon.

In considering the various pathways of VFA formation discussed,

a compartment model describing the flow of carbon from hexose to VFA can be constructed. This model is illustrated in Figure 11.

Efficiency of Volatile Fatty Acid Formation

The use of a stoichiometric fermentation balance approach in estimating the extent of ruminal fermentation has been suggested (Wolin, 1960) and discussed (Hungate, 1966; Ørskov et al., 1968; Ørskov, 1975). Theoretical amounts of VFAs, methane and CO_2 can be calculated from the number of moles of hexose fermented in each end-product. Estimates of the number of moles of hexose contributing to the production of each of the VFA can be calculated if the proportions of the VFAs produced are known. The latter can be measured through isotope dilution techniques (Bergman et al., 1965; Leng, 1970; Sutton, 1972) or estimated from the molar proportions of VFA present in the rumen. The latter method assumes that VFA production rates are proportional to their respective ruminal concentrations (Leng, 1970).

The stoichiometric equations to be used in computing a rumen fermentation balance are:

$$\text{hexose} + 2\ H_2O \longrightarrow 2\ \text{acetate} + 2\ CO_2 + 4\ H_2 \qquad (3.24)$$

$$\text{hexose} + 2\ H_2 \longrightarrow 2\ \text{propionate} + 2\ H_2O \qquad (3.25)$$

$$\text{hexose} \longrightarrow \text{butyrate} + 2\ CO_2 + 2\ H_2 \qquad (3.26)$$

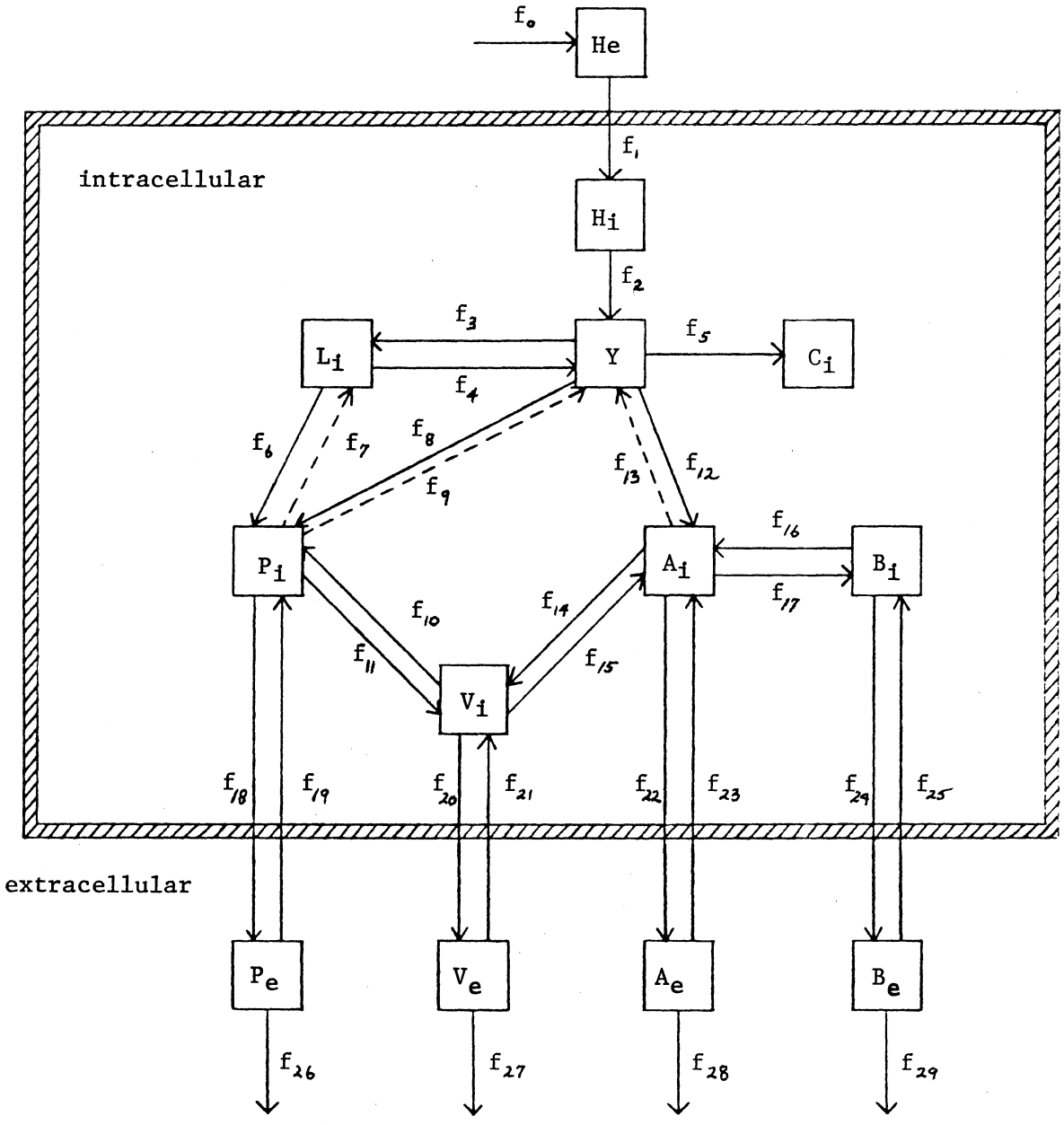
$$\text{hexose} + H_2 \longrightarrow \text{valerate} + CO_2 + 2\ H_2O \qquad (3.27)$$

$$\text{hexose} \longrightarrow 2/3\ \text{caproate} + 2\ CO_2 + 4/3\ H_2 + 2/3\ H_2O \qquad (3.28)$$

$$4\ H_2 + CO_2 \longrightarrow \text{methane} + 2\ H_2O \qquad (3.29)$$

Since 1 mole of hexose can produce 2 moles of acetate, or 2 moles

Figure 11. Simplified Compartment Model of VFA Formation from Hexose in the Rumen. Compartments indicated are: H- hexose, Y- pyruvate, L- lactate, A- acetate, B- butyrate, P- propionate, V- valerate, C- C_1 pool (CO_2, CH_4, formate). Flow of carbon between compartments is indicated by the f terms. Subscripts denote intracellular compartments (i) and extracellular compartments (e).


f_o
He
f_1
intracellular
Hi
f_2
f_3
L_i
Y
f_5
C_i
f_4
f_6
f_7
f_8
f_9
f_{13}
f_{12}
f_{16}
P_i
A_i
B_i
f_{17}
f_{10}
f_{14}
f_{11}
f_{15}
V_i
f_{18}
f_{19}
f_{20}
f_{21}
f_{22}
f_{23}
f_{24}
f_{25}
extracellular
P_e
V_e
A_e
B_e
f_{26}
f_{27}
f_{28}
f_{29}

of propionate, or 1 mole of butyrate, or 1 mole of valerate, or 2/3 mole of caproate, the proportions in which the hexose contributed to acetic, propionic, butyric, valeric and caproic acid formation will be, respectively, 1/2 A, 1/2 P, B, V, and 3/2 C. The A, P, B, V and C represent the molar proportions of acetic, propionic, butyric, valeric and caproic acid, respectively, that are produced (or present) in the rumen, such that

$$A + P + B + V + C = 1. \tag{3.30}$$

Hence, the number of moles of hexose fermented per mole of total VFA produced (H_x) is given by

$$H_x = 1/2\ A + 1/2\ P + B + V + 3/2\ C. \tag{3.31}$$

Equation (3.31) can be used to calculate the fraction of hexose fermented to each VFA. For example, the fraction of hexose converted to acetate (a) is given by

$$a = \frac{A}{2\ H_x}\ . \tag{3.32}$$

Similarly, the fraction of hexose converted to propionate, butyrate, valerate and caproate is given by

$$p = \frac{A}{2\ H_x}\ , \tag{3.33}$$

$$b = \frac{B}{H_x}\ , \tag{3.34}$$

$$v = \frac{V}{H_x} \tag{3.35}$$

and

$$c = \frac{3\ C}{2\ H_x}\ , \tag{3.36}$$

respectively, such that

$$a + p + b + v + c = 1. \tag{3.37}$$

The biochemical Equations (3.24) through (3.29) can be algebraically summarized using Equations (3.30) and (3.31) yielding

$$H_x \longrightarrow A + P + B + V + C + (M \bullet H_x) + (CD_n \bullet H_x) \tag{3.38}$$

where M is the number of moles of methane produced per mole of hexose fermented and CD_n is the net number of moles of CO_2 produced per mole of hexose fermented.

From Equations (3.24) - (3.29)

$$M = \frac{4a - 2p + 2b - v + 4/3\ c}{4}\ . \tag{3.39a}$$

Substituting Equations (3.32) - (3.36) into Equation (3.39a) and multiplying both sides by H_x yields

$$M \bullet H_x = \frac{2A - P + 2B - V + 2C}{4}\ . \tag{3.39b}$$

The net number of moles of CO_2 produced per mole of hexose fermented (CD_n) can be calculated by subtracting the number of moles of methane produced per mole of hexose fermented (M) from the total number of moles of CO_2 generated in the fermentation of one mole of hexose (CD_T). From Equations (3.24) - (3.29)

$$CD_T = 2a + 2b + v + 2c. \tag{3.40a}$$

Substituting Equations (3.32) - (3.36) into Equation (3.40a) and multiplying both sides by H_X gives

$$CD_T \cdot H_X = A + 2B + V + 3C. \tag{3.40b}$$

Subtracting Equation (3.39b) from (3.40b) gives an equation describing the net number of moles of CO_2 obtained during the formation of one mole of total VFA, such that

$$CD_n \cdot H_X = \frac{2A + P + 6B + 5V + 10C}{4} . \tag{3.41}$$

The loss of energy as heat during the fermentation of hexose, commonly termed the residual heat of fermentation (RHF), can be expressed as the difference in the heats of combustion of the products and reactant in Equation (3.38). Given the heat of combustion (kJ/mole) of hexose, acetate, propionate, butyrate, valerate, caproate and methane as 2816, 874, 1536, 2192, 2853, 3477 and 883, respectively, Equation (3.38) becomes

$$\begin{aligned}(2816)H_X \longrightarrow\ & (874)A + (1536)P + (2192)B + \\ & (2853)V + (3477)C + (833)M \cdot H_X + \\ & RHF \cdot H_X.\end{aligned} \tag{3.42}$$

From Equation (3.42) the RHF (kJ/mole of total VFA formed) can be calculated according to the following equation:

$$\begin{aligned}RHF \cdot H_X = (2816)H_X - [&(874)A + (1536)P + (2192)B + \\ &(2853)V + (3477)C + (883)M \cdot H_X].\end{aligned} \tag{3.43}$$

The fractional efficiency (E) of converting hexose energy to VFA energy can be calculated from Equations (3.31) and (3.42) where

$$E = \frac{0.621A + 1.091P + 1.557B + 2.027V + 2.470\ C}{A + P + 2B + 2V + 3C} . \qquad (3.44)$$

Several assumptions are involved in the approach just discussed (Ørskov et al., 1968; Ørskov, 1975). Briefly, these assumptions are:

(1) No hydrogen escapes the rumen as free hydrogen.

(2) The fermentation is strictly anaerobic.

(3) Reduction of sulfate, nitrate or unsaturated fatty acids is negligible.

(4) VFAs, CO_2, methane and water are the sole end products of fermentation. Lactate concentration is negligible. Hydrolysis of protein does not influence VFA production and branched chain VFAs result only from protein breakdown.

(5) VFA molar proportions are assumed proportional to their production rate, which may be a false assumption under certain feeding regimes (Leng, 1970).

(6) Incorporation of substrate into cellular material is assumed negligible; however, Hungate (1966) has estimated 10-12% of the substrate may be used in the synthesis of bacterial protoplasm. The significance of this observation may be more important at low rumen dilution rates (slow growth or resting state) than when cells are growing rapidly (Thompson and Hobson, 1971).

In Appendix B an example set of calculations is given to illustrate the utility of the fermentation balance approach in evaluating ruminal carbohydrate metabolism.

Absorption of Volatile Fatty Acids from the Rumen

The rumen is lined with a stratified, keratinized epithelium. Due to the generally non-absorptive nature of this type of tissue, it was thought for many years that the rumen was just a vessel for containing the fermentation. It was not until 1944 that the late Sir Joseph Barcroft and his colleagues at Cambridge, England (Barcroft et al., 1944), presented unequivocal evidence that volatile fatty acids are absorbed through the rumen wall. With the aid of Elsden's developments in partition chromatography (Elsden, 1946), the major VFAs were positively identified as acetic, propionic and butyric acids. The presence of C_4 and C_5 isomers and longer chain VFAs was shown by Gray et al. (1951).

Barcroft et al. (1944) noted a higher proportion of acetic acid in the blood draining the rumen than that present in the rumen fluid. This was attributed to an increased rate of absorption of acetic acid relative to the longer chain fatty acids. However, Kiddle et al. (1951) observed that the relative amount of butyric acid, compared to acetic acid, in the blood draining the sheep's rumen was less than the relative amount disappearing from the rumen, suggesting a utilization of butyrate by the rumen wall. Pennington (1952) demonstrated the ability of the rumen epithelial tissue to metabolize butyric acid and, to a lesser extent, acetic and propionic acids. It was later shown that isobutyric, valeric, isovaleric and 2-methylbutyric acids were also absorbed by the rumen wall (Annison and Pennington, (1954). Factors affecting the transport and metabolism of the VFAs

through the rumen epithelium have been reviewed recently (Stevens, 1970; Fell and Weekes, 1975).

The extent of metabolism of the VFAs by the rumen epithelium is unclear; however, butyrate is the most extensively metabolized, undergoing conversion to β-hydroxybutyrate and acetoacetate as well as oxidation via the TCA cycle (Pennington, 1954). The extent of butyrate metabolism is reported to vary from 50 to 90% of that absorbed (Stevens and Stettler, 1966; Bergman and Wolff, 1971; Weigand et al., 1975).

Bergman and Wolff (1971) and Leng et al. (1967) found that the rumen wall metabolized 50 to 70%, respectively, of the propionate absorbed. However, Weigand et al. (1972) and Weekes and Webster (1974) conclude that in vivo very little (5%) propionate is metabolized directly by the rumen wall. Their data suggests that lactate present in blood from the portal-drained viscera is derived via glycolysis occuring in the rumen epithelium from glucose which originally may have come from propionate in the liver. In vitro experiments suggest that the rumen mucosa converts 40-50% of propionate uptake to lactate (Weigand et al., 1975). The reason for these discrepancies is not clear, but may be related to glucose availability in the incubation medium.

Acetate utilization by the rumen epithelium is much less than that of butyrate (Cook and Miller, 1965). The utilization of acetate is thought to be limited by the low activity of acetyl-CoA synthetase (EC 6.2.1.1) in the rumen epithelium (Ash and Baird, 1973).

The rate of VFA absorption increases with increasing chain length (Danielli et al., 1945) and with a decrease in the pH of the rumen

contents. Absorption is also increased when the concentrations of the VFAs are increased (Stevens and Stettler, 1966). Stevens (1970) constructed a model of VFA absorption based on much of the available *in vitro* and *in vivo* experimental data. In the model, VFA absorption is primarily dependent on diffusion, but can be modified by the extent of VFA metabolism in the rumen epithelium. Also, the presence of pH and electrical gradients across the epithelial cell membrane may be important in VFA transport.

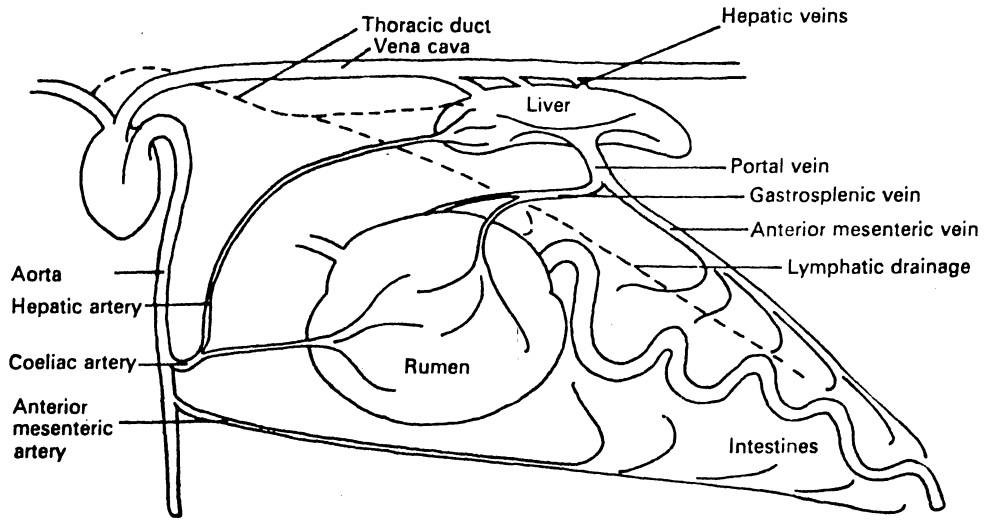
Figure 12 illustrates very schematically the circulation to the rumen, intestines and liver of the ruminant. Essentially all of the products of VFA absorption and metabolism in the rumen wall are cleared into the gastrosplenic vein before eventually draining into the portal vein. A very small proportion is drained by the lymphatic system into the thoracic duct (Webster, 1974).

VFAs entering the liver from the portal circulation are metabolized to varying degrees (Bergman, 1975). Acetate is not significantly metabolized by the liver presumably due to the low activity of acetyl-CoA synthetase (EC 6.2.1.1). On the other hand, liver metabolism of propionate and butyrate is essentially complete with only minute amounts (12 and 4 μM, respectively) appearing in the arterial circulation.

Utilization of Volatile Fatty Acids by the Ruminant Animal

Ørskov (1975) has indicated that the efficiency of food utilization by the ruminant is influenced, in at least three ways, by the type of rumen fermentation occuring at any given point in time.

Figure 12. Schematic Diagram of the Mesenteric and Hepatic Circulation in the Ruminant. Source: Webster (1974).


Thoracic duct
Vena cava
Hepatic veins
Liver
Portal vein
Gastrosplenic vein
Anterior mesenteric vein
Lymphatic drainage
Aorta
Hepatic artery
Coeliac artery
Anterior mesenteric artery
Rumen
Intestines

These are: (1) An effect on the efficiency of the fermentation; (2) An effect on the utilization of VFA by the host tissues; (3) An effect on the partition of dietary energy by the animal. The efficiency of rumen fermentation with respect to VFA production has been discussed in a previous section of this manuscript; therefore, a brief discussion of (2) and (3) will now be presented.

Metabolism of the VFA

The VFAs are absorbed, as previously described, through the rumen wall, resulting in a variable fraction of each VFA being metabolized before entering the portal circulation. Most of the VFA entering the portal circulation are cleared by the liver (Bergman and Wolff, 1971), with acetate as the only VFA reaching peripheral circulation in substantial quantities. Regulation of glucagon and insulin levels has been attributed to small amounts of propionate, butyrate or β-hydroxybutyrate reaching the peripheral circulation the first few hours postfeeding (Ross and Kitts, 1973); however, the physiological significance of this observation is doubted (Bassett, 1975).

Before being metabolized by the animal tissues, the VFAs must be activated through the formation of an acyl-coenzyme A intermediate. Consequently, the ability of various ruminant tissues to utilize the VFAs may be dependent on the relative activity of the thiokinases and thiophorases present in those tissues. A very brief discussion of the utilization of each VFA shall be presented.

Acetate. Acetate, instead of glucose, is the major substrate

for energy storage and oxidation in the ruminant, but it also participates in the synthesis of several body constituents (Blaxter, 1962; Baldwin, 1968). For efficient utilization of acetate, glucose or a glucogenic compound must be metabolized simultaneously (Blaxter, 1971). For example, the synthesis of long chain fatty acids requires reducing equivalents in the form of NADPH (reduced nicotinamide adenine dinucleotide phosphate) which are largely generated from glucose via the hexose monophosphate pathway. Hovell, as cited by Ørskov (1975), calculated that 1 mole of propionate was required for every 4.11 moles of acetate incorporated into long chain fatty acids.

Propionate. Propionate is an important precursor of glucose in ruminants, supplying as much as 50 to 60% of the carbon in glucose (Leng et al., 1967; Wiltrout and Satter, 1972).

Propionate metabolized to lactate by the rumen epithelium (Leng et al., 1967; Weigand et al., 1972) would decrease the amount of propionate available for gluconeogenesis. However, this lactate would also become a precursor for glucose in the liver. Therefore, the net contribution of propionate would not be diminished.

The conversion of propionate to TCA cycle intermediates involves an enzymatic step (methylmalonyl-CoA mutase) which requires vitamin B_{12} as a cofactor. Consequently, a sub-optimum level of available B_{12} in ruminant liver may alter the efficiency of propionate utilization (Garton et al., 1972; Ørskov, 1975). Normally, propionate is cleared quite efficiently by the liver, resulting in only traces appearing in the peripheral blood. However, the incorporation of an

intermediate of propionate metabolism, methylmalonyl-CoA, has been observed in significant quantities in the depot fat of lambs consuming high levels of barley concentrate diets (Garton et al., 1972). This indicates that the rate of propionate utilization in the liver may be exceeded under conditions of very high propionate flux or low B_{12} availability (Frobish and Davis, 1977).

Gluconeogenesis in the ruminant has been reviewed quite extensively by Ballard et al. (1969), Leng (1970b), Bergman (1973) and Young (1977). A brief review of glucose metabolism in ruminants will be found in a later section of this chapter.

Butyrate and Valerate. Butyrate is extensively metabolized to β-hydroxybutyrate by the rumen epithelium (Stevens and Stettler, 1966). In the liver, butyrate can yield 2 moles of acetyl-CoA or one mole of β-hydroxybutyrate. Similarly, catabolism of valerate can give 1 mole of acetyl-CoA and 1 mole of propionyl-CoA. These intermediates are metabolized as discussed previously with only trace amounts appearing in the extrahepatic circulation.

Utilization for Maintenance

Theoretical calculations (Baldwin, 1970; Blaxter, 1971) as well as experimental evidence (Armstrong et al., 1957) suggest that the energetic efficiencies of utilization of VFAs for maintenance are similar. However, Blaxter and co-workers (Armstrong et al., 1957; Blaxter, 1971) have shown that the efficiency of acetate utilization is markedly decreased if glucose or, more specifically, propionic acid availability is limited.

Ørskov (1975) calculated from the data of Armstrong and Blaxter (1957) that 1 mole of propionic acid was sufficient to ensure efficient oxidation and utilization of 16.3 moles of acetic acid. This calculation was based on the assumption that the ratio of C_2 carbon to C_3 carbon can be taken as a measure of the glucogenic energy required to efficiently metabolize acetic acid. Based upon the number of kJ required to result in the formation of ATP from ADP, the theoretically derived energetic efficiencies of acetate, propionate and butyrate relative to stearate are 89, 91 and 92%, respectively (Blaxter, 1971).

Although experimental evidence generally agrees favorably with these values, variation does exist. These discrepancies have been associated with an incomplete knowledge and understanding of the energetic transformations occuring in the various biochemical pathways involved in VFA utilization (Baldwin, 1970).

Inefficiencies in the utilization of VFAs for maintenance are reflected in the amount of heat produced after consumption of a diet at a level which is sufficient to spare body stores (maintenance level of intake). Baldwin (1970) refers to this as the heat increment of a feedstuff maintenance (HI_m). Therefore, the net amount of energy available to the animal for maintenance (NE_m) can be calculated by

$$NE_m = ME - HI_m \tag{3.45}$$

where ME is the metabolizable energy of a feedstuff. Efficiency of ME utilization for maintenance can be expressed as

$$E_m = \frac{NE_m}{ME} \quad . \tag{3.46}$$

Utilization for Growth and Fattening

Efficiencies of utilization of VFAs for tissue growth and/or energy storage (fattening) are largely dependent on the thermodynamic cost of the synthetic process involved. Information available about efficiency of energy utilization is generally with regard to synthesis of body fat. Information concerning growth or lactation is confounded by the variable contribution of body fat stores to the metabolic flux. Therefore, this makes the study of the partitioning of energy for various tissue functions quite difficult.

In the case of feedlot cattle, growth is associated with increases in protein as well as fat tissues. As the cattle progress through the feeding cycle, the relative amount of energy used for tissue synthesis shifts from protein synthesis to fat synthesis. Jessee et al. (1976b) found that gross energetic efficiency (Mcal empty body energy gained/ Mcal GE consumed) increased (13.0, 14.8 and 16.5%) as the slaughter weight of feedlot steers increased (341, 454 and 545 kg, respectively). This was attributed to fat deposition (fattening) being a more efficient process than protein deposition (growth). In addition, these investigators observed that an increase in the ratio of ground corn to corn silage from 30:70 to 80:20 resulted in an increased efficiency of energy gain from 13.6 to 16.8%, respectively. However, Jesse et al. (1976a) noted that NEg values did not differ ($P < .05$) as the ratio of ground corn to corn silage increased although the values were greatest for the high corn ration (80:20 corn to corn silage).

Although these studies suggest that the partitioning of dietary energy for different tissue functions may influence utilization efficiency, direct experimental evidence for this is lacking. Consequently, studies are needed to investigate the partitioning of dietary energy into protein and fatty tissues in feedlot steers, as influenced by the interactions of dietary protein level, level of microbial protein synthesis, biological value of protein absorbed, ratio of fermentation end-products and amount of carbohydrate absorbed post-ruminally.

An expression involving the efficiency of energy utilization of a feedstuff can be derived as previously developed for maintenance. The net energy value of a feedstuff for production (NE_p) is defined as

$$NE_p = ME - HI_p \tag{3.47}$$

where HI_p is the heat increment associated with production; i.e., tissue activity above that necessary for tissue maintenance. Therefore, the partial efficiency of ME utilization above maintenance (E_p) is

$$E_p = \frac{NE_p}{ME} . \tag{3.48}$$

Experimentally, HI_m and HI_p are not usually distinguishable; however, they reflect the variable energy cost of different physiological processes (Baldwin, 1970).

Generally, VFAs are used less efficiently above maintenance than below (Blaxter and Armstrong, 1957b; Armstrong et al., 1958; Blaxter, 1971). This is reflected in the difference between HI_m and HI_p

(Armstrong and Blaxter, 1957; Blaxter, 1962) where $HI_p > HI_m$. In practice, however, the differences in utilization efficiency are expressed as the NE_m and NE_p values of a feedstuff where $NE_m > NE_p$.

The efficiencies of utilization of the individual VFAs on a particular diet appear to be similar (Ørskov and Allen, 1966; Ørskov et al., 1966; Bull et al., 1970), while there seems to be an increase in the efficiency of acetate utilization associated with increasing the concentrate level of the diet (Ørskov and Allen, 1966; Poole and Allen, 1970). Presumably this is due to an increased absorption of glucogenic precursors.

Based on these and other observations, Ørskov (1975; 1977) has attempted to reconcile the variation in efficiency of VFA utilization by showing that VFA utilization depends on the ratio of non-glucogenic to potentially glucogenic precursors absorbed by the animal. He defines the non-glucogenic ratio (NGR) as the ratio of C_2 to C_3 carbon. Hence,

$$NGR = \frac{A + 2B + V}{P + V} \tag{3.49}$$

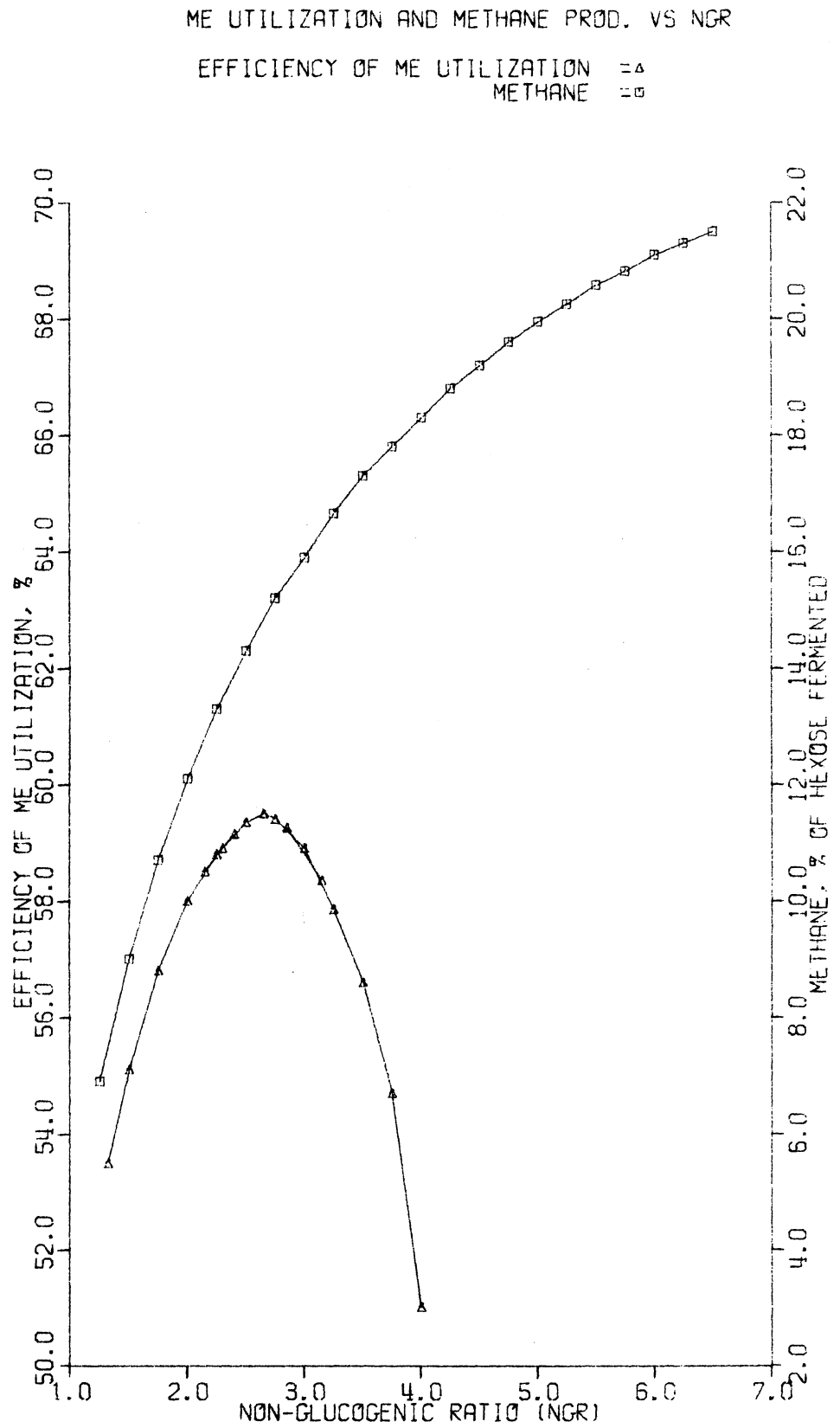
where the terms A, P, B and V represent the molar proportions of acetic, propionic, butyric and valeric acids, respectively. It is recognized that other non-glucogenic and glucogenic metabolites can arise from protein and lipid metabolism, but the main contributors to the NGR would be the fermentation end-products. This concept may need to be modified for diets which have been shown to have considerable passage of starch to the small intestine; i.e., diets containing high amounts of raw corn starch (Cole et al., 1976a). Glucose

absorbed post-ruminally could markedly affect the ratio of non-glucogenic to glucogenic carbon available for tissue metabolism.

Ørskov (1975) summarized several workers results and constructed a relationship of NGR to efficiency of ME utilization above maintenance. This relationship is illustrated in Figure 13. Using Equation (3.39b), the relationship between the NGR and the theoretical methane production as a percent of the quantity of carbohydrate fermented is also illustrated in Figure 13. An NGR of 2.25 - 3.0 would appear to give the best overall efficiency for fattening and growth. It is interesting to note that maximum efficiency of ME utilization does not necessarily occur with minimum methane formation. It is not known what effect post-ruminal absorption of glucose would have on these relationships, but it might be speculated that a higher NGR could be tolerated without decreasing the efficiency of ME utilization for production.

From the previous discussion it is apparent that the extent and type of rumen fermentation can alter the NGR and consequently the efficiency of ME utilization above maintenance. Since the majority of the energy absorbed by the ruminant is in the form of volatile fatty acids, the efficiency of their utilization is a primary determinant in the metabolic efficiency of the animal. Although the most important contributors to the NGR are the VFAs, this significance may be diminished somewhat when a substantial amount of energy is absorbed from the small intestine. For example, with steers consuming whole corn diets (Cole et al., 1976a), as much as 29% of the starch disappearing (feed-feces difference) was digested post-ruminally. Although this value includes the portion of starch fermented in the

Figure 13. Relationship Between NGR and Efficiency of ME Utilization for Fattening (Δ——Δ) and Theoretical Methane Production (□——□). NGR was calculated from Equation (3.49) and methane production was calculated by Equations (3.31), (3.39b) and (3.42). Calculations assume B = 0.1, V = 0.03 and C = 0.0. Source: Ørskov (1975).


ME UTILIZATION AND METHANE PROD. VS NGR
EFFICIENCY OF ME UTILIZATION =Δ
METHANE =□
EFFICIENCY OF ME UTILIZATION, %
METHANE, % OF HEXOSE FERMENTED
NON-GLUCOGENIC RATIO (NGR)
50.0
52.0
54.0
56.0
58.0
60.0
62.0
64.0
66.0
68.0
70.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0

large intestine, approximately 70% of the starch disappearing was digested in the rumen. Therefore, manipulation of rumen fermentation could be an important factor in maximizing food utilization. Ørskov (1975) has outlined and discussed some of the techniques currently being used toward achieving this goal. Briefly, these techniques involve:

(1) Varying the ratio of roughage to concentrate;

(2) Particle size optimization for certain feedstuffs;

(3) Level of feeding;

(4) Frequency of feeding;

(5) Optimizing the extent of processing of the concentrate;

(6) Varying the type of carbohydrate fermented;

(7) Development and use of improved feed additives;

(8) Inhibition of methane production.

Glucose Metabolism in Ruminants

Several excellent reviews concerning glucose metabolism in ruminants are available (Ballard et al., 1969; Leng, 1970b; Bergman, 1973; Young, 1977) while the reviews of Armstrong (1965) and Lindsay (1970) deal with the more general aspects of ruminant carbohydrate metabolism.

Although the plasma glucose concentrations in adult ruminants (400-700 mg/l) are lower than those found in non-ruminants (800-1100 mg/l), the ruminant's metabolic requirement for glucose does not appear to be drastically different than that of non-ruminants (Ballard et al., 1969). Glucose is the major energy source for ruminant brain tissue (O'Neal et al., 1966) and is required by the

mammary gland for milk lactose synthesis (Smith, 1971). In addition, during pregnancy the fetus demands glucose (Steel and Leng, 1973a) possibly due to lack of fetal lipoprotein lipase.

The primary substrate for fatty acid synthesis in the ruminant is acetate as contrasted with glucose for many nonruminants (Ballard et al., 1969). This has been attributed to the fact that several of the enzymes involved in lipogenesis from glucose are either absent or exist at very low levels. Specifically, these are glucokinase (EC 2.7.1.2; Ballard and Oliver, 1964), ATP citrate lyase (EC 4.1.3.8) and NADP-malate dehydrogenase (EC 1.1.1.40) in liver (Hanson and Ballard, 1967), and ATP citrate lyase, NADP-malate dehydrogenase and pyruvate carboxylase (EC 6.4.1.1) in adipose tissue (Hanson and Ballard, 1967). In adipose tissue, glucose is necessary for the synthesis of α-glycerophosphate used in triglyceride formation (Ballard et al., 1969).

Ballard et al. (1972) conducted studies where sheep were either fed a diet containing 70% soluble carbohydrate, abomasally infused glucose or received glucose by direct intravenous infusion. They found that in liver and adipose tissue ATP citrate lyase and NADP-malate dehydrogenase activities were increased due to increased availability of glucose suggesting that these enzymes are adaptable in both tissues. Glucose infusion increased lipogenesis in liver and adipose tissue from acetate, while in adipose tissue glucose became a quantitatively important precursor of triglyceride fatty acid. Consequently, substrate supply modifies enzyme levels and controls substrate use for lipogenesis.

As discussed previously, most of the dietary energy is fermented

in the rumen to volatile fatty acids, methane and CO_2. This situation dictates that the animal meet its glucose need through gluconeogenesis. In the liver, exogenous propionate and α-keto acids formed from the deamination of glucogenic amino acids are generally considered to be the two major precursors of glucose in ruminants. However, as mentioned earlier, feeding diets containing a high proportion of starch, particularly raw corn grain, may allow considerable amounts of dietary starch to escape ruminal fermentation and become available for absorption in the small intestine (Topps et al., 1968; Armstrong and Beever, 1969; Cole et al., 1976a). In addition, these types of diets usually give rise to an increased proportion of propionic acid in the rumen (Balch and Rowland, 1957). Both these factors would increase the amount of glucose or glucose precursors available to the animal.

Contribution of Exogenous Glucose to Glucose Entry Rate

Simultaneous measurement of intestinal starch digestion and glucose entry rate has not been conducted to date, however, several studies imply that ruminal bypass of starch and absorption of glucose in the small intestine increases the turnover rate of glucose. Increasing the dietary level of ground corn from 12 to 63%, without increasing DE intake, increased plasma concentration, pool size and turnover rate of glucose, and also decreased the half-life of the glucose pool (Evans and Buchanan-Smith, 1975). Increasing the DE intake to 2x maintenance also resulted in similar observations, which were concurrent with similar findings (Lindsay, 1970) that a

higher DE intake is correlated with a higher glucose turnover rate. This evidence, along with that of Buchanan-Smith et al. (1973), suggests that increased fatness of beef cattle fed diets containing a high proportion of grain is the result of a greater glucose availability. Most likely insulin is also involved, but its exact role and relative importance is unclear.

Judson and Leng (1973a) using sheep fed wheat or alfalfa diets found that intravenous infusions of glucose (4-144 mg/min) resulted in a suppression of endogenous glucose production (20-60% reduction) in direct proportion to the amount infused. They found that about 8% of the ingested wheat starch was absorbed as glucose from the small intestine and accounted for 34-44% of the irreversible loss of plasma glucose. The relative contribution of propionate to the synthesis of glucose decreased with increasing level of glucose infusion, again suggesting that an increase in the exogenous supply of glucose depressed gluconeogenesis. As expected, the irreversible loss of plasma glucose increased with increasing level of glucose infusion.

Judson et al. (1968) evaluated glucose turnover rates with three diets containing variable proportions of ground corn and alfalfa, but were unable to detect any differences between diets. They did find, however, that as the proportion of starch in the diet increased there was a reduction in the relative contribution of propionate to glucose synthesis. On the high roughage diet 46% of the propionate (molar proportion in the rumen VFA was 16%) produced in the rumen was converted to glucose, while on the high starch diet (molar proportion of 40%) only a 27% conversion was observed. Since the

production rate of propionate did not differ with diet, the authors suggested that an increase of starch digestion and absorption in the small intestine could account for these observations. These conclusions are supported by the work of Ulyatt et al. (1970) using diets containing varying amounts of starch.

From the findings presented above it is tempting to speculate that in ruminants fed at a constant level of DE intake and under a given physiological state (e.g. fattening) where the total metabolic requirement for glucose remains unchanged, that a shift in the site of starch digestion from the rumen to the small intestine may result in a more efficient utilization of the dietary energy. This increased efficiency would partly result from the energy saved due to the suppression of gluconeogenesis while the rest would probably result as an energy savings attributed to the bypassing of the ruminal fermentation. Although this hypothesis has been suggested by Black (1971) and Sutton (1971), experiments designed to directly test this aspect of ruminant carbohydrate digestion are desperately needed.

Contribution of Propionate to Glucose Entry Rate

The liver accounts for about 86% of the endogenous glucose production while the kidneys are responsible for the remaining 15% (Leng, 1970b). Propionate, produced in the rumen, is largely cleared from the portal blood in its passage through the liver (Bergman and Wolff, 1971) and probably makes an insignificant contribution to gluconeogenesis in the kidneys.

Metabolism of propionate by sheep-liver mitochondria occurs

solely by the methylmalonate pathway (Smith et al., 1967) via succinate oxidation in the TCA cycle. Transport of malate or PEP out of the mitochondria into the cytosol leads to the conversion of propionate-derived intermediates to glucose. Generally, it is assumed that ruminal propionate furnishes 50-60% of the carbon in glucose (Leng, 1970b; Bergman, 1973); however, as discussed earlier, this relative contribution can be influenced by the level of exogenous glucose absorption as well as a variable contribution of the glucogenic amino acids to gluconeogenesis.

Leng et al. (1967), using hourly fed sheep consuming an alfalfa hay diet, found that the incorporation of C-2 and C-3 of propionate into glucose indicated that 54% of both glucose and lactate synthesized arose from propionate carbon. Glucose entry rates were estimated from an intravenous primed infusion or continuous infusion of ^{14}C-labeled glucose, while propionate production and the incorporation of propionate carbon into glucose was determined using a continuous intraruminal infusion of specifically labeled ^{14}C-propionate. Considerable exchange of C-1 of propionate during the conversion into glucose occured. This was interpreted to represent extensive conversion of propionate to lactate in the rumen epithelium; however, their measurements did not preclude the possibility of propionate being converted first to glucose and then to lactate (Weigand et al., 1972). Approximately 32% of the propionate produced in the rumen was used for glucose synthesis.

Estimates of the amount of glucose derived from propionate in nonlactating and lactating dairy cows, consuming a 60:40 concentrate: alfalfa hay diet, indicated that 32 and 45%, respectively, of their

plasma glucose was obtained from ruminal propionate (Wiltrout and Satter, 1972). A correction for the crossing over of propionate carbon in the TCA cycle resulted in a maximum estimate of 60% for the contribution of propionate carbon to glucose synthesis during the lactating phase. An error in the determination of the production rate of propionate in the rumen prevented the calculation of the relative contribution of the propionate produced that was converted to glucose.

Judson et al. (1968) found that mature wethers fed varying proportions of ground corn and alfalfa had relatively less glucose being derived from propionate when the starch content of the diet increased. They found 36 to 56% of the plasma glucose was derived from ruminal propionate, although no differences were observed in ruminal propionate production rates. As mentioned earlier, a decrease in gluconeogenesis was suggested due to increased glucose absorption from the small intestine.

The effect of infusion of propionate into the rumen on the net rate of glucose synthesis from ruminal propionate was studied by Judson and Leng (1973b) using, simultaneously, an intravenous infusion of 6-^{3}H-glucose and an intraruminal infusion of 2-^{14}C-propionate. Sheep were given a daily ration of 800 g alfalfa in 24 equal portions at hourly intervals. Increasing the propionate production rate (0.43 - 3.08 mmole/min) resulted in an increase in the proportion of glucose synthesized from propionate (25 - 87%) while the proportion of propionate produced and converted into glucose (39-31%) remained practically unchanged. The rate of synthesis of glucose from propionate (15-85 mg/min) as well as the irreversible loss of plasma

glucose (61-97 mg/min) increased as propionate production increased. These observations suggest that an increased production of propionate results in an elevated rate of gluconeogenesis with a larger proportion of the glucose synthesized arising from propionate; however, the partitioning of the flux of propionate in the liver cell remained virtually unchanged. If an elevated rate of gluconeogenesis can be envisioned as an increase in the metabolic rate of liver cells, then this implies that a constant proportion of the propionate flux in the liver cell is used to replenish TCA cycle intermediates or other cellular metabolites (e.g. α-glycerophosphate). Furthermore, the availability of ruminal propionate appears to be a major determinant of the rate of glucose synthesis in sheep.

Steel and Leng (1973b) observed similar relationships in non-pregnant and pregnant sheep. Although rates of glucose synthesis were higher in pregnant sheep, the percentage contribution of propionate turnover to plasma glucose turnover was unchanged during pregnancy. The proportion of glucose derived from propionate varied between 19 and 62% and was dependent on the rate of production of propionate in the rumen in both pregnant and non-pregnant sheep. The proportions of ruminal propionate production converted into glucose were 46 and 56% in non-pregnant and 140 d pregnant sheep, respectively.

Table II summarizes a portion of the data obtained by various investigators with regard to the contribution of propionate to glucose synthesis.

TABLE II

SUMMARY OF EXPERIMENTS MEASURING CONVERSION OF PROPIONATE TO GLUCOSE IN RUMINANTS

Diet[a]	Feeding[b] Regime	Status[c]	Animal Wt. (kg)	Rumen Propionate mmole/min/kg	Production ($\times 10^2$)[d] mmole/min/kg$^{.75}$	Potential mg/min/kg	Glucose Synthesis[e] mg/min/kg$^{.75}$	Glucose mg/min/kg	Irrev. Loss[f] mg/min/kg$^{.75}$	Y_{gp}[h] (%)	Prop. Converted[i] to Glucose (%)	No. Expts.	Ref.[j]
800 g A	CC(12)	MT	37	2.5	6.2	2.3	5.6	1.6	4.0	54	38	2	1
800 g A	C(12)	MT	38	3.7	9.1	3.3	8.2	1.6	3.9	54	25	2	1
9.5 kg C:H (6:4)	C(24)	NL	660	1.3	6.4	1.1	5.8	1.4	7.3	32	41	4	2
17.0 kg C:H (6:4)	C(24)	L	660	2.6	13.3	2.4	12.0	2.7	13.5	45	50	4	2
800 g A	C(24)	1.4 x M	35	2.0	4.9	1.8	4.4	1.6[g]	3.8[g]	54	44	3	3
800 g A	C(24)	1.4 x M	36	1.7	4.1	1.5	3.8	1.6[g]	3.9[g]	41	44	6	4
500 g A:W (1:1)	H(24)	NP	33	1.1	2.5	1.1	2.3	1.3	3.2	30	42	4	5
500 g A:W (1:1)	H(24)	P(103)	33	1.1	2.6	1.0	2.3	1.5	3.7	34	53	4	5
500 g A:W (1:1)	H(24)	P(140)	35	0.7	1.8	0.6	1.6	1.8	4.3	23	60	3	5
800 g A	H(24)	NP	34	1.5	3.7	1.4	3.3	1.9	4.5	37	51	4	5
800 g A	H(24)	P(101)	36	1.7	4.2	1.5	3.8	2.1	5.2	41	54	4	5
800 g A	H(24)	P(143)	39	1.6	4.0	1.4	3.6	2.2	5.4	40	58	3	5
A	AL	NP	40	1.8	4.5	1.6	4.1	2.0	4.9	37	44	-	5
A	AL	P(99)	39	1.8	4.4	1.6	4.0	2.1	5.3	43	60	4	5
A	AL	P(138)	46	1.4	3.6	1.3	3.2	2.3	6.0	29	53	3	5

[a] A- alfalfa, C- concentrate, H- hay, W- wheat straw.

[b] AL- ad libitum, C- continuous, H- hourly, ( )- length of feeding period in hours.

[c] L- lactating, M- maintenance, MT- mature wt., NL- nonlactating, NP- nonpregnant, ( )- No. days pregnant.

[d] Determined from primed, constant ruminal infusion of 2-^{14}C-propionate.

[e] Assumes 2 mole propionate converted to 1 mole glucose.

[f] Determined by U-^{14}C-glucose intravenous infusion, unless otherwise noted.

[g] Determined by 6-^{3}H-glucose intravenous infusion.

[h] Transfer ratio of glucose carbon derived from propionate carbon x100.

[i] Percentage of propionate that is produced which is converted to glucose.

[j] References

1. Leng et al. (1967)
2. Wiltrout and Satter 1972)
3. Judson and Leng (1973a)
4. Judson and Leng (1973b)
5. Steel and Leng (1973b)

Contribution of Amino Acids to Glucose Entry Rate

Amino acid carbon is believed to be a main source of glucose carbon in ruminants. Almost all amino acids are glucogenic except lysine, leucine and taurine. Wolff and Bergman (1972) found that five amino acids (alanine, aspartate, glutamate, glycine and serine) supplied 11 to 30% of the carbon in glucose, of which about 80% was derived from alanine and glutamate. These amino acids have been proposed to function in alanine and glutamine cycles as an important means of linking amino acid metabolism in skeletal tissue with the gluconeogenesis occuring in the liver (Bergman, 1973).

Reilly and Ford (1971) demonstrated that the conversion rate of amino acid carbon into glucose was dependent on dietary protein intake. Using an intravenous infusion of ^{14}C-labeled amino acids, they estimated that the percentage of glucose carbon derived from plasma amino acids ranges from 20-30% in pregnant and non-pregnant sheep. In sheep receiving intra-abomasal infusions of enzymatic-hydrolyzed casein, Judson and Leng (1973b) observed an increase in gluconeogenesis and calculated that 13 g of additional plasma glucose was synthesized for every 100 g of casein hydrolysate infused.

The amount of bacterial protein synthesized during ruminal fermentation is correlated with the overall rate of VFA production in the rumen and makes up about 50% of the crude protein in digesta entering the small intestine (Hogan and Weston, 1970). Furthermore, the rate of growth of ruminal microorganisms appears to increase with increasing ruminal dilution rates (Hobson and Summers, 1967)

thereby increasing the flow of protein to the small intestine. Based on these relationships and the observations of Judson and Leng (1973b), an increased ruminal VFA production coupled with an elevated ruminal dilution rate could be expected to increase the supply of amino acids to the small intestine and possibly stimulate gluconeogenesis from amino acid carbon.

Contribution of Other Metabolites to Glucose Entry Rates

Glycerol can be used by the liver to synthesize glucose; however, in fed sheep the free glycerol concentration in blood is small and accounts for less than 5% of the glucose turnover. During starvation glycerol may become a more important glucose precursor increasing to as much as 40% of the glucose carbon being derived from glycerol (Bergman, 1973).

The contribution of lactate to the synthesis of glucose may be considered quite variable. In ruminants, unknown quantities of lactate can be absorbed from the rumen; however this is most likely on high concentration diets where the rumen microflora is poorly adapted to the feeding conditions (Hungate, 1966). As mentioned previously, lactate may be derived from propionate through metabolism in either the rumen mucosa or the liver. In addition, lactate may arise from certain amino acids such as alanine. Generally, lactate is originally derived from glucose metabolism in various body tissues (principally skeletal muscle) and recycled back to glucose in the liver or kidneys (Cori cycle). As a result, no net glucose can be synthesized from this recycling. In starvation this recycling may

be an important means of conserving glucose carbon.

Annison et al. (1963a) showed that 15% of the glucose pool came from lactate while 40% of the lactate originated from glucose in fed and fasted sheep. Their results were interpreted to suggest that a substantial portion of the lactate entry rate reflects the rate at which lactate equilibrates with plasma glucose. Using specifically labeled glucose, they observed that approximately 10% of the glucose carbon disappearing was recycled to glucose.

Glucose Turnover in the Whole Body

The kinetics of glucose metabolism in ruminants has been investigated quite extensively (White et al., 1969; Steel and Leng, 1973a; Judson and Leng, 1973a; Horsfield et al., 1974). Techniques used include both the single-dose method and the continuous-infusion methods of isotope administration. Similar estimates for glucose turnover are obtained from either method if proper mathematical analysis is followed (White et al., 1969; Hetenyi and Norwich, 1974). The isotope dilution data obtained from these experiments have been analyzed using multi-exponential analysis (White et al., 1969), multi-compartmental analysis (Leng, 1970b; Horsfield et al., 1974) or single pool kinetics (Judson and Leng, 1973a; Steel and Leng, 1973a; Evans and Buchanan-Smith, 1975). A complete discussion of these various techniques will not be presented here although a general treatment of the mathematical analysis involved was presented in Chapter II of this manuscript. The interested reader is referred to the discussions of Katz and Dunn (1967), White et al. (1969) and Leng (1970b).

Generally, a constant infusion of uniformily labeled ^{14}C-glucose

is used to determine glucose entry rate; however, the calculated entry rate usually underestimates the glucose production rate by 4-10% due to a recycling of carbon through lactate and other intermediates. This is also observed in single injection experiments using U-^{14}C-glucose. Leng (1970), using a single injection of a mixture of U-^{14}C-glucose and 3-^{3}H-glucose, observed that the ^{14}C/^{3}H ratio of plasma glucose remained unchanged up to 60 minutes post-injection. Therefore, recycling was not appreciable during this time period and a single compartment analysis was applied to this data. However, this probably represents only a first approximation of the glucose turnover rate since the glucose pool has been identified to contain at least three (White et al., 1969) or four major compartments (Horsfield et al., 1974). It should be noted that the single injection technique has been considered less precise due to problems with instantaneous mixing and mathematical calculation difficulties (Bergman, 1973).

Problems arising from the recycling of carbon have been essentially circumvented through the use of 2-^{3}H-glucose as a tracer for studying glucose metabolism (Katz and Dunn, 1967). Tritium from the C-2 position of glucose is lost via the rapid isomerization of hexose-6-phosphates and becomes rapidly equilibrated with water, therefore negligible quantities can be expected to be recycled to glucose. Tritium from 3-^{3}H-glucose is lost in a similar manner; however, this equilibration occurs during the triose isomerase step of glycolysis.

In studies where single pool kinetics are employed, the isotope is assumed to equilibrate with a glucose carbon pool (glucose pool) which includes the plasma glucose compartment and the interstitial fluid glucose compartment as well as the compartments of glucose

precursors equilibrating with this carbon pool. The space or volume of body tissue which contains this carbon pool is referred to as the glucose space. Pool size and turnover rate are calculated assuming single compartment kinetics.

Table III summarizes some of the data obtained by various investigators with regard to glucose turnover.

Kleiber (1967) has argued that when comparisons of metabolic activity (such as glucose turnover) are being made between animals whose weights are greatly different (i.e. sheep versus cattle), then pool sizes should be adjusted according to the body weight of the animal while turnover rates should be compared on the basis of metabolic weight or weight to the three-fourths power. Therefore,

$$f_{ii} \alpha W^{0.75} \tag{3.50}$$

and

$$Q_i \alpha W \tag{3.51}$$

where W is the weight of the animal.

Rearrangement of Equation (2.60) gives

$$k_{ii} = \frac{f_{ii}}{Q_i} , \tag{3.52}$$

which suggests, from Equation (3.50) and (3.51), that the turnover rate constant is proportional to the negative fourth root of body weight (Kleiber, 1975); i.e.,

$$k_{ii} \alpha W^{-0.25} . \tag{3.53}$$

TABLE III

SUMMARY OF EXPERIMENTS MEASURING PLASMA GLUCOSE TURNOVER AND RELATED PARAMETERS

Diet[a]	Feeding[d] Regime	Status[e]	Isotope Method[f]	Isotope Label[g]	Animal Wt. (kg)	Plasma Conc. (mg/l)	Pool Size (g)	Pool Size (mg/kg)	Space (% Wt)	Irreversible Loss mg/min/kg	Irreversible Loss $mg/min/kg^{.75}$	Turnover Rate Constant ($\times 10^2$) min^{-1}	Turnover Rate Constant ($\times 10^2$) $min^{-1} \cdot kg^{.25}$	Recycling Rate mg/min/kg	Ref.[h]
800 g A	C(12)	1.4 x M	CI	UC	37	680	-	-	-	1.6	3.9	-	-	-	1
800 g A	C(12)	1.4 x M	PI	UC	35	720	-	-	-	1.7	4.0	-	-	-	1
800 g A	C(12)	1.4 x M	SI	UC	37	680	4.5	122	18	1.6	3.9	1.6	4.1	0.41	2
800 g A	C(12)	1.4 x M	PI	UC	35	660	5.4	154	24	1.6	3.8	-	-	-	2
800 g A	C(12)	1.4 x M	CI	UC	37	680	-	-	-	1.7	4.2	-	-	-	2
800 g A	D	1.4 x M	SI	UC	34	790	4.1	121	16	1.9	4.5	2.3	5.6	0.91	2
100 g CP, 85% B	C(24)	1.4 x M	PI	UC	40	577	5.6	140	25	1.2	3.1	-	-	-	3
136 g CP, PGr	C(24)	1.4 x M	PI	UC	40	584	7.0	175	30	1.5	3.7	-	-	-	3
76 g CP, PH	C(24)	1.4 x M	PI	UC	44	575	5.1	116	20	1.4	3.5	-	-	-	3
595 g DOM, A	AL	NP	SI	UC	42	710	5.1	121	17	2.4	6.1	2.3	5.8	0.38	4
595 g DOM, A	AL	P(80)	SI	UC	36	700	5.6	156	22	2.2	5.4	1.6	3.9	0.28	4
595 g DOM, A	AL	P(100)	SI	UC	39	710	5.6	144	20	2.2	5.4	1.7	4.3	0.28	4
595 g DOM, A	AL	P(140)	SI	UC	41	660	5.4	132	20	2.6	6.7	2.5	6.3	0.71	4
575 g DOM, WC	H(12)	NP	SI	UC	34	790	5.5	162	20	2.6	6.2	2.2	5.2	0.91	4
405 g DOM, A	H(12)	NP	SI	UC	37	680	4.5	122	18	1.6	3.9	1.6	4.1	0.41	4
405 g DOM, A	H(12)	P(80)	SI	UC	33	660	4.1	124	19	1.8	4.3	1.8	4.3	0.45	4
405 g DOM, A	H(12)	P(106)	SI	UC	32	670	4.5	141	21	2.0	4.8	1.9	4.5	0.66	4
405 g DOM, A	H(12)	P(140)	SI	UC	34	610	4.3	126	21	2.3	5.5	2.4	5.7	0.68	4
235 g DOM, A:WS (1:1)	H(12)	NP	SI	UC	31	670	5.0	161	25	1.7	4.1	1.3	3.0	0.35	4
235 g DOM, A:WS (1:1)	H(12)	P(80)	SI	UC	32	620	4.8	150	24	1.5	3.5	1.1	2.7	0.22	4
235 g DOM, A:WS (1:1)	H(12)	P(100)	SI	UC	28	510	3.6	129	25	1.8	4.0	1.8	4.1	0.50	4
235 g DOM, A:WS (1:1)	H(12)	P(140)	SI	UC	33	460	4.2	127	28	1.8	4.2	1.7	4.1	0.39	4
Starvation	-	S(0)	SI	UC	37	680	4.5	122	18	1.6	3.9	1.6	4.1	0.41	4
Starvation	-	S(2)	SI	UC	31	550	2.8	90	17	1.0	2.4	2.7	6.3	1.4	4
Starvation	-	S(4)	SI	UC	31	590	3.7	119	20	0.77	1.8	1.2	2.9	0.71	4
Starvation	-	S(6)	SI	UC	36	520	3.6	100	19	0.8	1.9	1.6	3.7	0.77	4
Starved 4 d	-	P(80)	SI	UC	36	470	3.9	108	23	1.1	2.8	1.3	3.2	0.31	4
Starved 4 d	-	P(100)	SI	UC	38	450	3.6	95	21	1.2	3.0	1.5	3.8	0.24	4
Starved 4 d	-	P(140)	SI	UC	40	310	2.5	63	20	1.1	2.8	2.3	5.8	0.35	4
63% GC	2D	1 x M	SI	2H	34	613	4.0	119	19	2.0	4.9	1.7	4.1	-	5
63% GC	2D	2 x M	SI	2H	41	769	6.2	152	20	3.0	7.7	2.0	5.1	-	5
12% GC	2D	1 x M	SI	2H	41	515	4.0	98	19	1.5	3.9	1.5	3.8	-	5
12% GC	2D	2 x M	SI	2H	41	553	4.4	108	20	1.9	4.9	1.8	4.6	-	5
[b]12% CP Starter	AL	NP-CW	SI/PI	3H/UC	86	1120	26.3	306	27	1.4	4.3	0.71	2.2	0.78	6
[c]Alaskan Pasture, My'69	G	NP-CW	SI	3H	81	530	8.5	105	20	1.0	3.1	0.99	3.0	-	6
[c]Alaskan Pasture, Au'69	G	NP-CW	SI/PI	3H/UC	104	1290	35.1	338	26	1.7	5.5	0.92	2.9	1.42	6
[c]Alaskan Pasture, Ja'70	G	P-LW	SI/PI	3H/UC	94	1190	18.9	201	16	0.55	1.7	0.9	2.8	1.25	6
[c]Alaskan Pasture, Ap'70	G	P-LW	SI/PI	3H/UC	90	980	21.5	239	22	0.92	2.8	0.93	2.9	1.31	6

TABLE III (Continued)

[a]A- alfalfa, B- barley, CP- crude protein, DOM- digestible organic matter, GC- ground corn, PGr- ground, pelleted dried grass, PH- ground, pelleted hay, WC- fresh cut white clover, WS- wheat straw.

[b]Penned Reindeer fed Purina Cattle Starter II, 12% crude protein.

[c]Reindeer grazing native Alaskan pasture during season indicated.

[d]AL- ad libitum, C- continuous, D- once daily, G- grazing, H- hourly, 2D- twice daily, # h- length of feeding period in hours.

[e]CW- constant weight, LW- losing weight, M- maintenance, NP- nonpregnant, P- pregnant, S- starvation, # d- number of days.

[f]CI- constant infusion, PI- primed, constant infusion, SI- single injection.

[g]UC- uniformily labeled ^{14}C glucose, 3H- 3-^{3}H-glucose.

[h]Reference

1. Leng et al. (1967)
2. White et al. (1969)
3. Ulyatt et al. (1970)
4. Steel and Leng (1973a)
5. Evans and Buchanan-Smith (1975)
6. Luick et al. (1973)

It follows then that when comparing sheep and cattle, the turnover rate constants for a particular metabolic process should be adjusted according to Equation (3.53). A correction factor (CF) for adjusting turnover rate constants in a sheep (k_s), with a body weight of W_s, to turnover rate constants in a steer (k_c), with a body weight of W_c, can be derived. Hence,

$$k_c = k_s \frac{W_s^{0.25}}{W_c^{0.25}} . \tag{3.54}$$

From Equation (3.54), the correction factor becomes

$$CF = \frac{W_s^{0.25}}{W_c^{0.25}} = \left(\frac{W_s}{W_c}\right)^{0.25} \tag{3.55}$$

It should be kept in mind that such adjustments assume the animals are in a similar physiological status; i.e., similar heat production per unit metabolic weight. Furthermore, adjustment based on the metabolic size of the animal may not be valid for turnover rate constants of pools not directly associated with the energy turnover of the animal.

Production of Volatile Fatty Acids in the Rumen as Measured by Isotope Dilution

The biochemistry of VFA production in the rumen has already been discussed in a previous section of this chapter. This section will primarily be concerned with presenting some of the experimental results of investigators using the isotope dilution technique to measure VFA production in ruminants.

Techniques and General Assumptions

Several experimental methods are available, both *in vitro* and *in vivo*, for estimating VFA production in the rumen. For a review of these methods, the reader is referred to the article by Warner (1964) who has excellently evaluated and reviewed these various techniques. The isotope dilution procedure appears to be the most popular *in vivo* method for measuring VFA production (Sutton, 1972).

Although the single injection method has been used, the preferred method is the primed dose, constant infusion experiment. When the single dose method has been used, first-order kinetics have been employed (Sheppard *et al.*, 1959; Gray *et al.*, 1960; Davis, 1967; Knox *et al.*, 1967). This is likely to be in error because a significant reversible flow of carbon occurs between the acetate and butyrate compartments (Bergman *et al.*, 1965; Gray *et al.*, 1965; Leng and Leonard, 1965). Because of this recycling of carbon, VFA production, as calculated by Equation (2.62), must be corrected to obtain net production rates. This has been accomplished by the use of non-compartmental (Gray *et al.*, 1965; Leng and Leonard, 1965) and compartmental (Bergman *et al.*, 1965; Libby, 1974) analysis.

The isotope dilution technique recently has gained the acceptance of most investigators due to its relative simplicity of use and accuracy of measurement (Sutton, 1972). The technique was developed by Gray and his colleagues (Gray *et al.*, 1960) and has been extensively used in the study of VFA production in sheep consuming various forage diets (Sheppard *et al.*, 1959; Gray *et al.*, 1960, 1965, 1966, 1967; Leng and Leonard, 1965; Leng and Brett, 1966; Leng *et al.*, 1968;

Bergman et al., 1965; Weller et al., 1967; Weston and Hogan, 1968; Faichney, 1968; Weller et al., 1969). VFA production in dairy cows, as determined by isotope dilution, has been investigated (Davis, 1967; Knox et al., 1967; Esdale et al., 1968; Wiltrout and Satter, 1972); however, studies in beef cattle, especially under feedlot conditions, are lacking. Recently, the production and interconversion rates of acetate and butyrate have been estimated in an in vitro continuous culture of rumen microorganisms (Libby, 1974).

A major criticism of the isotope dilution procedure is that in the rumen, mixing may be slow and that representative samples are difficult to obtain due to the heterogeneity of rumen contents. Also, measurements of VFA production in animals fed once or twice daily may not be validly extrapolated over the entire 24 hour cycle, since daily production rates may vary with frequency of feeding (Sutton, 1972). The results of Gray et al. (1967) do not support this proposition since they found daily VFA production rates to not vary in sheep fed roughages at intervals of 1, 2 or 12 hours, even though production rates were not constant immediately after feeding (Gray et al., 1965). The rumen VFA production system is probably 'damped' when fermenting roughages, however when fermenting concentrates it displays a more 'transient' response. Therefore, it is quite likely that when feeding concentrates daily VFA production rate does vary with frequency of feeding (Sutton, 1972).

In several studies, the problems just mentioned have been practically alleviated or at least diminished appreciably by feeding an equal portion of the diet at hourly intervals (Leng, 1970a). Although it may be argued that results obtained under such steady-

state conditions are not applicable to situations where animals are fed once or twice daily, it probably does represent a valid simulation of the feeding behavior in ruminants under grazing or feedlot conditions.

Relationship of VFA Concentration to VFA Production Rate

One of the main results from the work done with sheep consuming forage diets is that VFA production rates are generally proportional to their corresponding ruminal VFA concentrations (Gray et al., 1966; Weller et al., 1967; Weston and Hogan, 1968). Leng (1970) concluded that this may not always be true, but any error incurred in assuming it to be so would be negligible. However, these assumptions are based on observations in ruminants consuming forage diets and consequently may not be valid when ruminants are fed high-concentrate diets (Leng and Brett, 1966; Esdale et al., 1968).

The concentration of a particular VFA in the rumen at any time can be expressed as a function of its net production rate, absorption rate, rumen dilution rate, rumen volume and any other factor which may directly or indirectly affect these variables. Hence, the apparent linearity observed between concentration and production rate with forage diets is probably related to the narrow range of operating conditions (within a local neighborhood around an operating point) under which the rumen ecosystem is exposed. However, when the system is subjected to more varying levels of external stimuli, such as that found with high-concentrate diets, the non-linearities inherent to most biological systems will probably be expressed.

VFA Production in Sheep

Single Dose Method. Sheppard et al. (1959) injected 1-^{14}C-acetate (20 minutes after feeding) into the rumen of a sheep consuming an all-clover hay ration. Assuming first-order kinetics, they calculated the turnover rate for acetate as 14.3%/hr; however, in their calculations they erroneously used $\log_{10}$ instead of $\log_e$. Applying the appropriate correction factor (2.303) gives a turnover rate of 33.0%/hr. Further analysis of their data reveals that the specific activity-time curve for acetate is not first-order, but can be described as the sum of two exponential decaying functions. This probably reflects the reversible flow of carbon between acetate and butyrate (Bergman et al., 1965). Incorporation of acetate carbon into butyrate as well as propionate and higher acids was observed.

Assuming a two compartment model comprised of the acetate and butyrate compartments, single dose analysis of the data yields turnover rate constants (k_{ii}) of 55 and 40%/hr for acetate and butyrate, respectively. This succinctly illustrates the magnitude of difference that can be obtained when applying single pool kinetics to a multi-compartment system. They estimated the size of the acetate compartment to be 342 mmoles. However, during the experimental period there was a marked decline in VFA concentration, indicating non-steady state conditions. Therefore, the validity of estimates made under such conditions are questionable.

Gray and his colleagues at Adelaide have conducted several studies investigating VFA production in sheep consuming diets containing finely chopped wheat straw and/or alfalfa hay. In one study

(Gray et al., 1960), acetate, propionate or butyrate (each labeled at the C-1 position) was injected into the rumen of a sheep consuming chopped wheat straw. After allowing time for mixing, specific activity of the acids was measured at suitable time intervals over a 5 hour period. Decline in specific activity obeyed first-order kinetics giving turnover rate constants of 45, 55 and 55%/hr for acetate, propionate and butyrate, respectively. They observed interconversions of carbon between all three acids, but it was more extensive between acetate and butyrate.

It should be pointed out that in these experiments the decline in specific activity was monitored for only 5 hours following injection of the isotope, while in the experiments of Sheppard et al. (1959) it was followed for 12 hours. This could explain why only first-order kinetics were observed by Gray et al. (1960) since the 'slow' component of Sheppard et al. (1959) specific activity-time curve did not manifest itself until after 5 hours.

Gray et al. (1965), using isotope dilution, compared VFA production in an artificial rumen (Gray et al., 1967) to that observed in vivo. The sheep were fed a diet consisting of 75% wheat straw and 25% alfalfa, which was given in equal portions twice daily. Acetate, propionate or butyrate labeled in the C-1 position or propionate labeled in the C-2 position was injected into either the rumen or artificial rumen as a single dose. Decline of specific activity was monitored for 3 hours. Experiments with the artificial rumen found that most of the label (95%) was recovered in the three acids except when 1-^{14}C-propionate was used as tracer. Presumably the C-1 of propionate was decarboxylated and lost as CO_2, decreasing

the recovery of label to approximately 80%. Based on these observations, subsequent studies used 2-^{14}C-propionate as tracer for the propionate compartment.

Turnover rate constants calculated for the VFA compartments in the artificial rumen were approximately 65% of those measured in vivo. The turnover rate constants for acetate, propionate and butyrate were 19.4, 16.3, 15.7 and 11.9, 10.4, 10.7%/hr for the rumen and artificial rumen, respectively. These observations are in agreement with that of others comparing VFA production in vivo to that obtained in vitro (Hungate, 1966).

A summary of VFA production in sheep as determined by the single dose method, is given in Table IV.

Constant Infusion Method. Leng and Leonard (1965), in one of the first studies to apply the constant infusion technique to the measurement of VFA production in the rumen, measured VFA production rates in sheep consuming 75 g per hour chopped alfalfa hay. Single infusions, lasting up to 4 hours, of C-1 labeled acetate, propionate or butyrate were performed. From the plateau specific activity values and infusion rates, production rates (P_i) for each acid were calculated according to Equation (2.62). Recognizing interconversion of the VFA carbon, they calculated 'effective production rates' according to the equation

$$EP_i = P_i - \sum_{j=1}^{n} P_j \, Y_{ji} \qquad (3.56)$$

where Y_{ji} is the transfer ratio for carbon in compartment i to carbon

TABLE IV

SUMMARY OF VFA PRODUCTION AS DETERMINED BY THE SINGLE DOSE ISOTOPE DILUTION METHOD

Diet[a]	Feeding[b] Regime	Animal[c] Wt. (kg)	Isotope[d]	Molar Percent[e] Ac	Pr	Bu	V	TVFA[f] mmole/l	Rumen[g] Status	Label[h] Kinetics	Turnover Rate Constant (%/hr) k_a	k_p	k_b	Transfer Ratio x100[i] Y_{ab}	Y_{ba}	Y_{ap}	Y_{pa}	Y_{bp}	Y_{pb}	Pool Size (mmoles) Ac	Pr	Bu	No. Expts	Ref.[j]
CH	2D	64	A1	72.6	17.5	7.3	2.6	91.9	NS	SO	55	-	40	-	12	-	5	-	-	342	-	-	1	1
800 g WS	AL	S	A1,P1,B1	67.0	19.4	13.6	-	61.2	SS	FO	45	55	55	-	-	-	-	-	-	197	57	40	3	2
WS:A (3:1)	AL	S	A1,P2,B1	71.6	16.7	11.7	-	84.1	NS	FO	19	16	16	-	-	-	-	-	-	-	-	-	1	3
8.2 kg A:10.9 kg G	3D	497	A1	64.7	20.2	11.8	3.3	109.0	SS	FO	51	-	-	-	-	-	-	-	-	2390	-	-	4	4
2.3 kg A:15.0 kg G	3D	503	A1	46.4	37.9	10.6	5.2	120.6	SS	FO	58	-	-	-	-	-	-	-	-	2060	-	-	5	4
not specified	-	HC	AH,P2,B2	-	-	-	-	-	SS	FO	18	16	23	10	49	3	37	18	5	7580	2710	1340	3	5

[a] A- alfalfa, CH- clover hay, G- grain, WS- wheat straw.

[b] AL- ad libitum, 2D- twice daily, 3D- three times daily.

[c] HC- Holstein cow, S- sheep.

[d] A1- 1-^{14}C-acetate, AH- 3-^{3}H-acetate, B1- 1-^{14}C-butyrate, B2- 2-^{14}C-butyrate, P1- 1-^{14}C-propionate, P2- 2-^{14}C-propionate.

[e] Ac- acetate, Bu- butyrate, Pr- propionate, V- valerate.

[f] TVFA- total volatile fatty acid concentration.

[g] NS- nonsteady-state, SS- steady-state.

[h] FO- apparent first order, SO- apparent second order.

[i] Y_{ij}- transfer of label from pool j to pool i (Transfer ratio).

[j] Reference

1. Sheppard et al. (1959)
2. Gray et al. (1960)
3. Gray et al. (1965)
4. Davis (1967)
5. Knox et al. (1967)

in compartment j (see Chapter II). This is not the same as net production rate (f_{oi}), but provides a first approximation of the actual net production rate (Bergman et al., 1965). Their estimates of the production rates for acetate, propionate and butyrate are 4.23, 1.17 and 0.97 mmoles per min, respectively, while estimates for the corresponding effective production rates are 3.85, 1.01 and 0.64 mmoles/min. Interconversion of acetate and butyrate were lower than that found by others (Bergman et al., 1965; Weller et al., 1967) with only 17% of the butyric acid carbon arising from acetate in comparison to 61% as determined by Bergman et al. (1965). Later studies by Leng and Brett (1966) found 40-50% of the extracellular butyric acid carbon was derived from extracellular acetate.

Weller et al. (1967) using single infusions of a labeled VFA in sheep concluded that the molar ratio of the VFAs corresponded to the relative rates of VFA production. Based on their experimental observations they assumed that the net production rate of each acid could be calculated from the total VFA net production rate and the molar ratio of each acid present. Furthermore, all the necessary calculations could be derived from data obtained from the infusion of only one labeled acid since any one labeled acid could result in the labeling of all other acids.

First, they calculated the specific activity of the total VFA pool (a_T) as

$$a_T = \sum_{i=1}^{n} a_{ie} M_{fi} \qquad (3.57)$$

where M_{fi} is the molar fraction of acid i and a_{ie} is the specific activity of acid i at steady-state (μCi/mole). The total VFA net production rate (P_T) can be calculated using Equation (2.62) yielding

$$P_T = \frac{u_i}{a_T} . \tag{3.58}$$

Now, the net production rate of each acid can be calculated as

$$f_{oi} = P_T M_{fi} . \tag{3.59}$$

It should be emphasized that these calculations yield estimates of net production rates and not gross production rates (P_i). Weller et al. (1967) applied this algorithm to the data of Bergman et al. (1965) and were able to derive the same values for net VFA production rates that Bergman and his colleagues obtained through compartmental analysis of the same data.

Weston and Hogan (1968) found VFA production in sheep consuming varied diets of ryegrass and forage oats to be highly correlated with the total amount of organic matter digested (r = 0.93-0.95). Approximately 56.8 g of VFA were produced per 100 g of organic matter digested. Isotope dilution experiments confirmed the observations of Weller et al. (1967) and Leng and Brett (1966) concerning the relationship between VFA production rates and VFA molar proportions. Furthermore, they calculated that 76% of the VFA produced in the rumen was absorbed from the rumen, 19% was absorbed from the omasum and abomasum, and 5% passed from the abomasum to the intestines.

Bergman et al. (1965) were the first investigators to apply

compartmental analysis toward the measurement of ruminal VFA production. Measurements were made in sheep consuming a dried grass cube diet fed at a continuous rate. Primed dose, constant infusions of 1-^{14}C-acetate, 2-^{14}C-propionate or 1-^{14}C-butyrate were used to label the VFA compartments.

A three compartment model, illustrated in Figure 14, was assumed to describe the VFA production and interconversion system. They claimed that the solution of the model could be obtained by solving 10 simultaneous equations. However, it is difficult to see how 10 linearly independent equations can be obtained from a three compartment model. Close inspection of their equations reveals that Equation (3) is the same as Equation (10), the only difference being the units in which the equations are written. Likewise, it can be shown that Equation (2) is identical to Equation (7) and Equation (1) is the same as Equation (4). Therefore, the 10 equations used by Bergman et al. (1965) can be reduced to 7 linearly independent equations. Although 9 linearly independent equations can be written for a three compartment model (assuming that entry into all compartments is possible), only 7 linearly independent equations are necessary to obtain a unique solution to the incomplete model described by Bergman et al. (1965).

If their data are used in Equations (2.37-2.46), the calculated model parameters are the same as those reported. These parameters are listed in Table VI, while a comparison of the net production rates with the gross production rates can be found in Table V. Gross production rates were calculated using Equation (2.62) and agree well with the values calculated by incorporating the appropriate

Figure 14. Three Compartment Model of VFA Production in the Rumen. Symbols in the figure are: A,P,B represent the acetate, propionate, and butyrate compartments, respectively. Flow rates (f) represent carbon transfer into, out of or between the compartments as indicated in the figure. Source: Bergman *et al*. (1965).

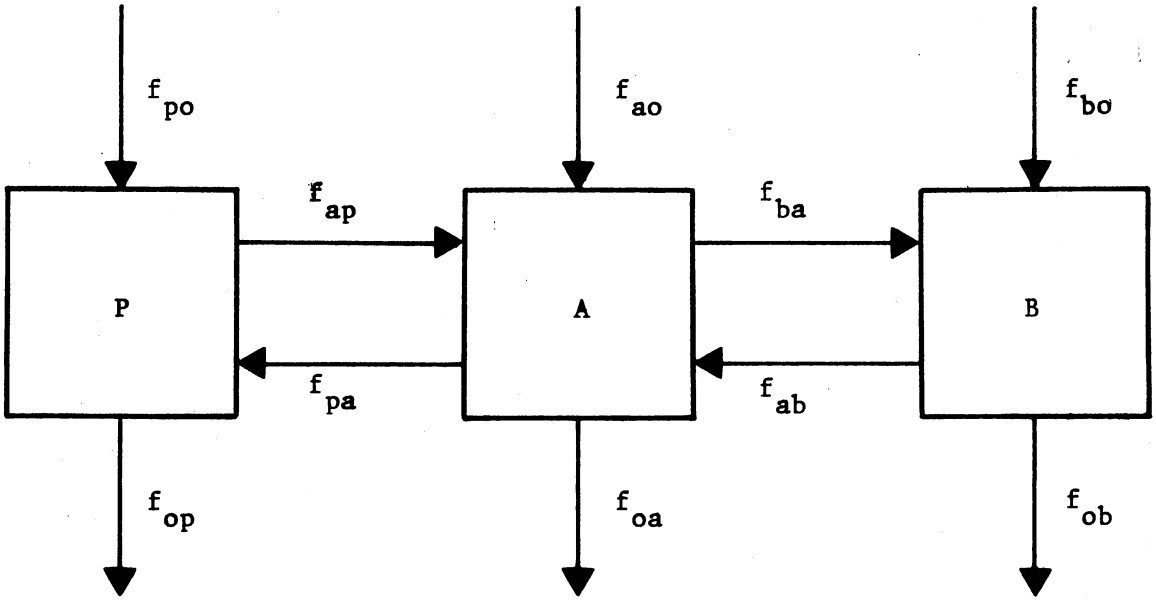

f_{po}
f_{ao}
f_{bo}
f_{ap}
f_{ba}
P
A
B
f_{pa}
f_{ab}
f_{op}
f_{oa}
f_{ob}

TABLE V

SUMMARY OF VFA PRODUCTION AS DETERMINED BY THE CONSTANT INFUSION ISOTOPE DILUTION METHOD

Diet[a]	Feeding[b] Regime	Animal[c] Weight kg	Isotope[d]	Molar Percent				Total[e] VFA mmole/l	VFA Production Rate (mmole/min) Gross[f]			Net[g]			Transfer Ratio x100[h]						Pool Size[i] mmoles			No. Expt	Ref.[j]
				Ac	Pr	Bu	V		P_a	P_p	P_b	F_a	F_p	F_b	Y_{ab}	Y_{ba}	Y_{ap}	Y_{pa}	Y_{bp}	Y_{pb}	Ac	Pr	Bu		
900 g GC	C(24)	54	Al,P2,Bl	68	19	13	-	105.7	3.26	0.854	0.729	2.57	0.729	0.472	20	61	4.5	14.0	4.8	4.8	397	122	81	12	1
900 g A	H(12)	37	Al,P1,Bl	69	20	9	1.6	123.0	4.23	1.170	0.970	3.85	1.010	0.640	15	17	3.2	4.5	0.9	3.4	365	106	37	13	2
500 g A:WS (6:4)	2D	S	Al,P2,Bl	68	18	14	-	-	2.71	0.598	0.782	2.16	0.528	0.479	11	86	2.0	26.0	7.6	7.3	-	-	-	7	3
800 g A	H(12)	37	AU,PU,BT	68	18	8	5.7	131.3	4.25	0.980	0.550	3.70	0.980	0.290	11	47	0.0	3.9	0.0	3.1	426	113	53	6	4
400 g C, 200 g A	H(12)	31	AU,PU,BT	59	22	13	6.2	112.9	3.00	0.750	0.500	2.55	0.740	0.320	12	44	0.2	6.0	0.2	2.6	262	98	58	6	4
300 g C, 300 g A	H(12)	30	AU,PU,BT	62	21	12	5.6	73.2	2.6	0.640	0.420	2.28	0.580	0.250	13	48	1.4	4.3	2.3	2.9	156	53	30	5	4
450 g WS, 50 g A	H(12)	29	AU,PU,BT	75	18	5	2.6	68.2	1.35	0.340	0.150	1.23	0.340	0.110	6	40	0.2	2.7	0.3	2.0	250	60	17	6	4
Pasture	G	41	AU,PU,BT	67	18	10	5.0	91.3	1.91	0.528	0.428	1.75	0.528	0.352	11	38	-	-	-	-	-	-	-	9	5
3.9 kg A	H(24)	JC	Al,P2,Bl	71	16	10	3.0	77.2	16.00	3.680	2.570	13.60	3.610	1.320	18	62	1.0	12.0	1.0	5.0	4280	963	602	6	6
3.5 kg CS	H(24)	JC	Al,P2,Bl	62	17	16	5.0	82.6	19.40	4.790	4.240	13.60	4.650	3.190	15	72	1.0	14.0	1.0	9.0	3780	1160	1030	6	6
9.5 kg G:A (6:4)	H(24)	660(NL)	Al,P2,Bl	63	18	17	2.0	117.0	35.70	5.340	7.850	28.30	3.810	5.950	15	63	8.0	17.0	7.0	10.0	-	-	-	8	7
17.0 kg G:A (6:4)	H(24)	660(L)	Al,P2,Bi	58	30	11	1.0	123.0	56.00	18.300	10.300	40.90	13.800	7.500	15	63	8.0	17.0	7.0	10.0	-	-	-	8	7

[a] A- alfalfa, C- corn, CS- corn silage, G- grain, GC- grass cubes, WS- wheat straw.

[b] C- continuous, 2D- twice daily, G- grazing, H- hourly, ( )- length of feeding period in hours.

[c] JC- Jersey cow, L- lactating, NL- nonlactating, S- sheep.

[d] Al- 1-^{14}C-acetate, Pl- 1-^{14}C-propionate, P2- 2-^{14}C-propionate, PU- 2-^{14}C-propionate, Bl- 1-^{14}C-butyrate, BT- 2-^{3}H-butyrate.

[e] Total volatile fatty acid concentration.

[f] Calculated according to Equation (2.62).

[g] Effective production rate for ref. 2, 4, 5.

[h] Y_{ij}- transfer of label from pool j to pool i.

[i] Calculated from VFA concentration and rumen liquid colume.

[j] Reference

1. Bergman et al. (1965)
2. Leng and Leonard (1965)
3. Weller et al. (1967)
4. Leng and Brett (1966)
5. Leng et al. (1968)
6. Esdale et al. (1968)
7. Wiltrout and Satter (1972)

TABLE VI

PARAMETERS OF BERGMAN THREE COMPARTMENT VFA MODEL CALCULATED FROM VARIOUS LITERATURE SOURCES

Diet[a]	Feeding[a] Regime	Animal[a] Weight	VFA Turnover Rate[b] g-atoms of carbon/hr										Pool Size (Q)[c] g-atoms of carbon			Turnover Rate Constant[d] %/hr									Ref.[e]
		kg	F_{aa}	F_{oa}	F_{pa}	F_{ba}	F_{pp}	F_{op}	F_{ap}	F_{bb}	F_{ob}	F_{ab}	Ac	Pr	Bu	k_{aa}	k_{oa}	k_{pa}	k_{ba}	k_{pp}	k_{op}	k_{ap}	k_{bb}	k_{ob}	k_{ab}	
900 g GC	C(24)	54	0.451	0.305	0.0242	0.1220	0.154	0.138	0.0159	0.199	0.110	0.0887	0.794	0.366	0.324	56.8	38.4	3.05	15.40	42.1	37.7	4.34	61.4	34.0	27.40	1
900 g A	H(12)	37	0.522	0.433	0.0484	0.0406	0.210	0.194	0.0157	0.239	0.163	0.0764	0.730	0.318	0.148	71.5	59.3	6.63	5.56	66.0	61.0	4.94	161.0	110.0	51.60	2
3.9 kg A	H(24)	JC	2.180	1.630	0.1080	0.4400	0.660	0.643	0.0168	0.697	0.315	0.3820	8.560	2.890	2.410	25.5	19.0	1.26	5.14	22.8	22.2	0.58	28.9	13.1	15.90	3
3.5 kg CS	H(24)	JC	2.570	1.580	0.1680	0.8180	0.858	0.835	0.0227	1.140	0.774	0.3660	7.560	3.480	4.120	34.0	20.9	2.22	10.80	24.7	24.0	0.65	27.7	18.8	8.88	3
9.5 kg G:A (6:4)	H(24)	660	4.710	3.400	0.2490	1.0600	1.010	0.681	0.3290	2.100	1.420	0.6730	-	-	-	-	-	-	-	-	-	-	-	-	-	4
17.0 kg G:A (6:4)	H(24)	660	7.490	4.910	0.7510	1.8300	3.230	2.710	0.5240	2.870	1.800	1.0700	-	-	-	-	-	-	-	-	-	-	-	-	-	4

[a] See Table V.

[b] F_{ij}- Flow of carbon from compartment j to compartment i, F_{ii}- total turnover rate of compartment i, F_{oi}- net production rate of compartment i.
Subscripts: a- acetate, b- butyrate, p- propionate, o- outside of system.

[c] Ac- acetate, Bu- butyrate, Pr- propionate.

[d] $k = F/Q$.

[e] Reference
1. Bergman et al. (1965)
2. Leng and Leonard (1965)
3. Esdale et al. (1968)
4. Wiltrout and Satter (1972)

terms from the model in Equation (2.61).

Comparisons of the production rates and turnover rates for the individual VFAs indicate that failure to correct production rates for the interconversion (recycling) of carbon to other acids can result in a gross overestimation of the total amount of VFAs being produced and available for absorption. Bergman et al. (1965) found that the total production rate (ΣP_i = 6.98 moles/d) was approximately 22% higher than the total net production rate (Σf_{oi} = 5.43 moles/d). Considerable interconversion between acetate and butyrate was present, with 61% of the butyrate carbon in equilibrium with 20% of the acetate carbon. A substantial portion of the digestible energy intake was fermented to VFAs since the net production of VFAs accounted for 62% of the digestible energy intake.

Table V summarizes some of the experimental results of researchers using the constant infusion method to determine VFA production rates in sheep.

VFA Production in Cattle

Isotope dilution experiments to determine VFA production in the bovine rumen have been almost exclusively conducted with dairy cattle.

Single Dose Method. Davis (1967), investigating milk-fat depression in lactating Holstein cows, measured acetate production by injecting a single dose of 1-^{14}C-acetate into the rumen. The cows were fed either a control diet (8.2 kg alfalfa hay, 10.9 kg grain supplement) or a high-grain diet (2.3 kg alfalfa hay, 15.0 kg grain supplement). The daily grain supplement was fed in three

equal portions and consisted of 75% ground corn, 12.5% crushed oats, 10% soybean meal and 2.5% minerals. Cows consuming the high-grain diet experienced a 50% depression in the level of milk-fat.

The decline in specific activity of acetate was measured for 10 hours following injection of label and their data indicated that a first-order process could describe acetate turnover. The acetate turnover rate constants from their data yields values of 51.0 and 58.2%/hr for the control and high-grain diets, respectively. Values for the acetate pool size were 2.39 and 2.06 moles for the cows fed the control and high-grain diets, respectively. Therefore, the respective acetate production rates in cows fed the control and high-grain rations are 29.3 and 28.8 moles/d.

Acetate and butyrate interconversions were observed, although the values were not reported, and it was noted that acetate conversion to butyrate was increased on the high-grain diet. Acetate production rates were corrected for acetate and butyrate interconversions giving net production rates of 25.1 and 21.8 moles/d for the control and high-grain diets, respectively. This is to be compared to the acetate concentrations of 70 and 55 mmoles/l in the rumen of cows fed the control and high-grain rations, respectively.

In another experiment (data not given) propionate production rate on the high-grain diet was found to be 24 moles/d. The high-grain diet had a significantly lower ($P < .01$) acetate:propionate ratio (1.27) than did the control diet (3.32).

Knox et al. (1967), using single dose experiments with ^{3}H-acetate, 2-^{14}C-butyrate and 2-^{14}C-propionate in a Holstein cow, found turnover rate constants of 18, 16 and 23%/hr for the acetate,

propionate and butyrate pools, respectively. The transfer of label between acetate and butyrate indicated that 49% of the butyrate pool was in equilibrium with 10% of the acetate pool; however, this may be misleading since different isotopes were used. The transfer ratio for acetate to propionate (0.37) was exceptionally high and may be due to an error associated with using ^{3}H-labeled acetate.

A summary of VFA production in dairy cattle as measured by the single dose method is given in Table IV.

Constant Infusion Method. Esdale et al. (1968) determined VFA production in a nonlactating Jersey cow fed hourly either alfalfa hay (3.9 kg DM/d) or corn silage (3.5 kg DM/d). Continuous isotope infusions (8-10 hr duration) of 1-^{14}C-acetate, 1-^{14}C-butyrate or 2-^{14}C-propionate were made. When the cow was fed the hay ration, 62% of the butyrate carbon was in equilibrium with 18% of the acetate carbon, but when fed the corn silage ration 72% of the butyrate carbon was derived from acetate while only 15% of acetate came from butyrate. This supports the earlier observation by Davis (1967) that including grain in the ration increases the conversion of acetate to butyrate.

The net production rates of acetate, propionate and butyrate with the silage ration were 19.6, 6.7 and 4.6 moles/d while with the hay ration they were 19.6, 5.2 and 1.9 moles/d, respectively.

Wiltrout and Satter (1972) examined VFA production in lactating and nonlactating Holstein cows consuming 17.0 and 9.5 kg DM/d, respectively, of a 60% concentrate:40% alfalfa hay diet. Continuous ruminal infusions of 1-^{14}C-acetate, 2-^{14}C-propionate or 1-^{14}C-

butyrate were conducted, but an 'error' in the propionate infusions prevented a direct measurement of propionate production rate. Transfer ratios indicated considerable interconversion of acetate and butyrate with 63% of the butyrate carbon in equilibrium with 15% of the acetate carbon. It was also noted that 37 and 23% of the valerate carbon was derived from acetate and propionate, respectively.

Net daily production rates for acetate and butyrate in nonlactating cows were 42 and 8 moles/d, while in lactating cows these rates shifted to 77 and 6 moles/d. A crude estimate of propionate production from the concentration of propionate in the rumen yielded 12 and 25 moles/d during the nonlactating and lactating phase, respectively.

Net daily production rates of the VFAs were calculated using the 10 simultaneous equations reported by Bergman et al. (1965). However, if the specific activities and infusion rates reported by Wiltrout and Satter (1972) are used in Equations (2.37-2.46), large discrepancies are obtained between the model parameters obtained (see Tables V and VI) and those reported. Wiltrout and Satter (1972) noted that their calculations yielded unrealistic propionate production values and this was assumed to be an 'error' in the infusion of labeled propionate. However, as noted earlier, the 10 equations of Bergman et al. (1965) are not linearly independent. Therefore, it is conceivable that erroneous values could be obtained from certain data sets even if the data are valid and 'error' free. Values for the model parameters, as calculated by Equations (2.37-2.46), are given in Table V and Table VI. These values (Table V)

seem quite realistic when compared to those of Esdale et al. (1968).

Using the three compartment model (Figure 14) of Bergman et al. (1965) and Equations (2.37-2.46), model parameters were calculated from the data sets of Leng and Leonard (1965), Esdale et al. (1968) and Wiltrout and Satter (1972). These values, along with those of Bergman et al. (1965) are presented in Table VI.

Liquid Turnover in the Rumen

The rate of liquid flow from the rumen to the omasum can be estimated from the exponential decline of the concentration of a water soluble, non-absorbable marker when that marker is added to the rumen as a single dose (Hyden, 1961). Single compartment analysis yields a linear relationship between the natural logarithm of marker concentration and time. Therefore,

$$\ln [M_c(t)] = \ln [M_o] - k_m t \tag{3.60}$$

where $M_c(t)$ is the concentration of marker at time t, M_o is the marker concentration at t=0 (obtained by extrapolation) and k_m is the turnover rate constant for the marker. The volume of rumen liquid into which the marker has diffused can be calculated as

$$V_m = \frac{D_m}{M_o} \tag{3.61}$$

where D_m is the amount of marker injected into the rumen at t=0.

The volume of distribution of a water soluble marker in the rumen is assumed to represent the volume of water contained therein. Faichney (1975) recently reviewed the use of markers in measuring

the flow of digesta through the gastro-intestinal tract of ruminants. He indicated that the preferred water soluble markers for rumen liquid are ^{51}Cr-EDTA and polyethylene glycol (PEG, molecular weight 4000). Hyden (1961) reports that 95% of the ruminal water volume in sheep and goats is equilibrated with PEG. The cellular water compartment of the ingested plant material (nonviable cells) is presumably labeled by PEG (Hungate et al., 1971). The extent of equilibrium is not known; however, the concentration of PEG is probably much less than that in extracellular water. PEG does not enter the microbial cells (viable) or the ruminant cells lining the gut, but it may, under some conditions, become associated with a fraction of the particulate matter in rumen digesta (Neudoerffer et al., 1973).

The turnover or flow (f_m) of fluid from the rumen to the omasum can be expressed as

$$f_m = k_m V_m \ . \qquad (3.62)$$

Although this is assumed to represent the flow of fluid through the reticulo-omasal orifice, it may not truly represent the actual flow rate due to incomplete mixing of saliva with the entire rumen contents (Engelhardt, cited by Faichney, 1975).

It is clear from Equation (3.62) that the turnover of the rumen liquid compartment will be affected by factors influencing the turnover rate constant or volume or both. Probably the most important variable affecting liquid turnover is the level of feed intake (Sutton, 1971b). Increasing feed intake of a hay-concentrate diet

from 3 to 9 kg dry matter per day resulted in increases in the turnover rate constant from 4.55 to 12.5%/hr (Poutiainen, cited by Sutton, 1971b). In addition, an increase in dry matter intake will usually result in an increase in rumen volume. Putnam et al. (1966) observed that in steers consuming a 25% hay ration, rumen volume increased approximately 50% when intake was doubled.

Other factors may also influence the turnover of rumen liquid. Physical form of the diet has been shown to affect rumen turnover. Under conditions of equal dry matter intake, Putnam et al., (1966) found greater rumen volumes in steers consuming an 89% hay ration (51 l) versus those consuming a 25% hay ration (40 l). They also noted an increased saliva flow in steers on the 89% hay diet as compared to those on the 25% hay ration. This may be a reflection of an increase in the amount of time spent ruminating since saliva flow has been shown to increase with rumination (Bailey and Balch, 1961). Ørskov et al. (1974) noted that rumination time in sheep fed whole barley diets (402 min/d) was almost double that found in sheep fed a pelleted barley diet (216 min/d). In animals fed rations consisting primarily of finely ground grains, rumination is markedly decreased or absent (Freer and Campling, 1965). Consequently, a much lower level of saliva flow may occur in animals being fed high-concentrate diets containing large amounts of finely ground grain (Balch, 1958; Lawlor et al., 1966) as opposed that which may be present in animals consuming high-concentrate diets composed chiefly of unprocessed grains.

When rumen volume is held constant, saliva flow is probably a primary factor in determining the magnitude of the liquid compartment

turnover rate constant. This is probably of major importance in feedlot cattle since it has been demonstrated that inclusion of a small amount of roughage in the diet results in significant improvement in animal performance when compared to that obtained when the animals are fed an all-concentrate ration (Weichental and Webb, 1969; White et al., 1975). This improvement may not be due entirely to an increase in saliva flow (i.e., turnover) since detrimental pathological changes in the rumen epithelial tissues are prevented or greatly decreased by inclusion of roughages in all-concentrate diets (Vance et al., 1970).

Under conditions of continuous feeding, rumen volume remains fairly constant (Murray et al., 1962). Therefore, assuming that feeding conditions in the feedlot are reasonably simulated by continuous feeding, one might conclude that inclusion of a 'roughage factor' in the diet of feedlot cattle would increase the turnover of rumen liquid as compared to that occuring under all-concentrate conditions. All-concentrate diets composed chiefly of whole grains have been shown to have a 'roughage value' when compared to all-concentrate rations containing finely ground or pelleted grains (White et al., 1972; Ørskov et al., 1974).

An increase in rumen liquid turnover can increase the net yield of nutrients to the host in at least two ways (Hungate, 1966). First, the increased turnover or dilution rate effectively lowers the concentration of the bacterial population present in the rumen and allows them to assume a new growth rate which is a function of the new feed rate (i.e., flow of fermentable material per unit time). As the mean specific growth rate of the bacterial population increases,

there is a tendency for the fermentation to proceed through less efficient pathways (i.e., less ATP produced/mole substrate fermented); however, the rate of ATP generation per unit time is increased to support the increased growth rate (Hobson, 1965). Undoubtly shifts in the bacterial population occur resulting in a selection of those species which can maximize ATP generation per unit time while maintaining a high specific growth rate. As a result, a higher proportion of the energy flux through the bacterial population is utilized for growth and therefore an improved efficiency for microbial growth is realized. Isaacson et al. (1975), using continuous mixed cultures of rumen microorganisms, found that at a low dilution rate (2.0%/hr) approximately 55% of the energy derived from glucose was used for maintenance of the bacterial population, while at a higher dilution rate (12.0%/hr) only 15% of the energy was used for maintenance. Therefore, an increased rumen turnover can result in an increased outflow of bacterial cells which are available to the host for digestion and absorption post-ruminally. This increased output is primarily in the form of microbial protein (Cole et al., 1976b); however, Isaacson et al. (1975) noted that at higher dilution rates the relative amount of carbon in the microbial cells increased approximately 5% which would suggest an increase in microbial polysaccharide storage.

Another beneficial effect of an increased rumen turnover would be an increase in the outflow of undigested portions of the feed. High concentrate diets are rich in readily fermentable materials of which starch is a major portion. Once the starch has been released from the grain (by mastication, both initially or during rumination,

and enzymatic and hydrolytic mechanisms), its rate of disappearance is a combination of digestion and rate of passage. Due to its relatively slower rate of degradation, as compared to soluble sugars, a larger proportion will escape ruminal digestion as turnover of the liquid is increased. As a result, a greater amount of α-linked glucose polymers is available for digestion and absorption in the lower tract.

From the previous discussion, it should be apparent that an increased rate of rumen turnover can result in a greater quantity of glucogenic and potentially gluconeogenic substrates being available post-ruminally to the host. As discussed earlier, when the host derives a larger proportion of its daily energy needs from this source, an improvement in metabolic efficiency should be realized. If the effect is not offset by a proportional decrease in rumen fermentation as influenced by an increased rumen turnover and nutrient bypass, then the net effect will be an increased energy and nitrogen supply to the host. This may explain part of the improvement in performance observed in feedlot steers consuming a whole corn high-concentrate ration when compared to that seen in steers being fed a finely ground corn high-concentrate ration (Gerken et al., 1971; Vance et al., 1972).

Feeding Value of Whole Versus Ground Dry Shelled Corn in High Concentrate Rations

This review will conclude with a summary of selected studies in which animal performance and various rumen parameters have been observed in beef steers consuming high-concentrate diets containing

either whole or ground dry shelled corn. Only a synoptic overview will be presented, since this subject has been recently reviewed by Rounds (1973) and Cole (1975).

Animal Performance

The performance of an animal is usually evaluated in two ways: (1) average daily weight gains (ADG), and (2) feed efficiency (FE). The latter of these is usually expressed in terms of the number of units of feed required to obtain one unit of ADG.

Most of the research with high-concentrate diets containing dry shelled corn supports the idea that feeding whole corn produces animal gains and feed efficiencies that are superior to those obtained from feeding mechanically processed (rolled, cracked or ground) corn. Henderson and Geasler (1971) have summarized the results of 13 different trails conducted at five university experiment stations (Indiana, Iowa, Michigan, Minnesota, Ohio) on the effect of grinding, rolling or cracking dry shelled corn on ADG of feedlot steers. When rates of gain were compared in steers consuming whole dry shelled corn, ADG was increased by grinding, rolling or cracking of dry shelled corn (1.23 and 1.17 kg/hd/d on processed and whole corn, respectively) if the corn made up less than 70% of the ration. On the other hand, whole shelled corn stimulated rate of gain when shelled corn made up 80% or more of the ration (1.11 and 1.07 kg/hd/d on whole and processed corn, respectively).

In studies with all-concentrate dry shelled corn rations, Weichental and Webb (1969) found a 5% increase in ADG and a 7% improvement in FE when whole corn was compared to ground corn.

When 10% roughage was added to the diets, gains were similar on the whole corn and ground corn rations; however, steers consuming the whole corn diet were 7% more efficient in converting feed dry matter to live weight gain. Similar observations have been reported by Hixon et al. (1969), Vance et al. (1970), Gerken et al. (1971), Vance et al. (1972) and White et al. (1975a,b).

Steers fed under ad libitum conditions generally show higher feed intakes on whole corn high-concentrate diets than when fed high-concentrate ground or cracked corn diets (Vance et al., 1972; White et al., 1975a,b). In a paired feeding study comparing 84% dry shelled corn rations, Rounds (1973) reported that intakes recorded for steers consuming a finely ground corn ration limited the intake of steers consuming a similar ration containing whole corn. Perhaps part of the superiority of whole corn feeding may be explained by an increase in feed intake. Site and extent of digestion could also be important (Cole, 1975).

Digestibility of Dry Matter and Starch

Dry matter digestibility (DMD) appears to be unaffected by physical form of the corn at low roughage levels. White et al. (1972) reported DMD in steers consuming all-concentrate dry shelled corn rations of 90.0 and 90.2% for the ground and whole corn rations, respectively. In rations containing 7% roughage as cottonseed hulls (CSH), Rounds (1973) reported DMD of 80.9 and 82.0% in steers consuming whole and ground dry shelled corn, respectively. Cole et al. (1976a) found DMD significantly higher ($P < .05$) in steers consuming an all-concentrate whole shelled corn ration when compared to that

in steers consuming whole shelled corn rations containing 7% roughage as CSH (84.3 vs 78.4% DMD, respectively). Adding 20% rice straw to a whole shelled corn all-concentrate ration depressed ($P < .05$) DMD from 86.5 to 69.3% (White et al., 1975b).

Digestibility of ground corn starch in the total tract of cattle approaches 98-99% (Karr et al., 1966; Waldo et al., 1971) but feeding whole corn has resulted in reduced starch digestibility. Cole et al. (1976a) found total starch digestibilities of 96.4 and 94.7% for 90 and 82% whole corn rations, respectively. Increasing particle size (Adeeb et al., 1971) and/or the presence of a horny endosperm (Beever et al., 1970) could diminish the availability of the raw corn starch for digestion.

Ruminal digestion of ground corn starch is higher in sheep (Tucker et al., 1968) than that found in cattle (Karr et al., 1966); however, corn source and level of corn in the ration are also important determinants of corn starch digestion in the rumen. Waldo (1973) reports a mean value of 74% for ground corn starch digestion in the rumen. Cole et al. (1976a) reported ruminal starch digestibilities of 90 and 82% from rations of 80 and 68% whole corn, respectively. This suggests that on high-concentrate whole shelled corn rations, particle size reduction via rumination is sufficient to maintain a ruminal starch fermentation similar to that occuring in steers fed ground corn high-concentrate diets.

The importance of rumination in the digestion of whole corn is supported by the findings of Nordin and Campling (1976) where studies using nylon bags containing whole, coarsely ground or finely ground corn were suspended in the rumen for varying time intervals. The

percentages of DM lost after 48 hours incubation were 19, 55 and 57% for the whole coarsely ground and finely ground corn, respectively. Adding 20% rice straw to a whole shelled corn ration increased ($P < .05$) the rate of passage of whole corn through the rumen as well as through the entire digestive tract (White et al., 1975b). Presumably this is due to an increase in rumination time and, subsequently, an increase in saliva flow. Rate of passage post-ruminally tended to increase and apparent digestibilities of energy, DM, crude protein and nitrogen-free extract decreased ($P < .05$) with the feeding of 20% rice straw.

Digestion of starch in the small intestine appears to be a function of the amount of starch flowing post-ruminally. Karr et al. (1966) found that digestibility of ground corn starch in the small intestine increased as the amount of starch entering the duodenum increased. Total daily starch digestion in the small intestine increased with increasing starch flow, but appeared to level off at an inflow rate of 9.7 $g/kg^{.75}$ (78% digested). Feeding an 82% whole corn ration resulted in a daily intestinal starch input of 11.6 $g/kg^{.75}$ of which 84% was digested in the intestines (Cole et al., 1976a).

Rumen pH and VFA Patterns

Rumen pH tends to be lower while total VFA concentrations tend to be higher with ground corn rations as compared to whole corn rations (Vance et al., 1970; Vance et al., 1972; White et al., 1972; White et al., 1975b). Feeding whole corn high-concentrate rations has generally been associated with higher acetate/propionate ratios;

however, fermentation patterns are usually similar to that found with ground corn (Vance et al., 1970; Vance et al., 1972; Rounds, 1973; White et al., 1975b). In contrast, other studies have reported feeding whole corn high-concentrate diets results in large acetate/propionate ratios similar to that found when forages are fed (White et al., 1972; Cole et al., 1976a). The reasons for this are not clear, but it may be related to an increased rumen dilution rate under conditions of whole corn feeding. Hodgson and Thomas (1975) demonstrated a negative relationship between the molar proportion of propionic acid and rumen dilution rate.

Vance et al. (1972) and White et al. (1975b) reported a higher proportion of valerate with whole corn as compared to crimped or ground corn, respectively. Utley and McCormick (1975) observed lower isovalerate proportions with a cracked corn, 20% peanut hull ration as compared to an all-concentrate whole corn diet.

Table VII summarizes several experiments examining rumen pH and VFA total concentration and molar proportions in steers consuming high-concentrate corn rations.

Conclusion

The preceeding review has covered only a small fraction of the total literature concerning ruminant digestion and metabolism. However, it should be apparent that ruminant digestion and metabolism is a highly complex and interactive process. Data concerning all aspects of this process are accumulating rapidly making the task of understanding and evaluating such a complex biological system in toto quite formidable. The development of valid mathematical models of

TABLE VII

pH, MOLAR PERCENT AND TOTAL CONCENTRATION OF VFAS IN THE RUMEN OF STEERS CONSUMING WHOLE OR GROUND CORN HIGH-CONCENTRATE RATIONS

Physical Form	% Corn	% Roughage	DM[a] Intake kg	pH	Total VFA mmole/l	VFA Molar Percent[b] Ac	Pr	Bu	IV	V	Ac/Pr	Ref.[d]
Whole	87.5	0	9.20(AL)	5.58	155.2	39.9	45.5	7.3	2.8	4.2	0.88	1
Whole	77.5	10	10.60(AL)	6.24	114.7	51.7	30.5	10.4	4.0	3.2	1.70	1
Ground	87.5	0	9.30(AL)	5.48	132.1	37.0	44.1	10.7	2.2	5.7	0.84	1
Ground	77.5	10	11.10(AL)	5.97	122.7	47.2	35.3	9.0	4.5	3.7	1.34	1
Whole	75.0	0	2.80(D)	6.20	254.6	53.5	29.8	10.8	2.4	2.0	1.80	2
Ground	75.0	0	2.80(D)	5.60	308.0	46.5	41.8	5.2	1.9	2.4	1.11	2
Whole	88.5	0	(AL)	5.60	110.3	38.6	40.5	13.2	2.5	5.3	0.95	3
Crimped	88.5	0	(AL)	5.50	124.2	43.6	39.5	10.0	3.0	4.2	1.10	3
Whole	84.0	7	6.37(H)	-	160.3	43.3	38.8	12.1	2.8	3.0	1.12	4
Ground	84.0	7	5.47(H)	-	153.5	39.2	39.8	16.8	1.2	3.0	0.99	4
Whole	100.0	0	5.58(AL)	6.20	117.8	52.8	31.2	9.8	1.2	3.7	1.69	5
Ground	100.0	0	4.62(AL)	6.10	124.0	50.2	34.4	10.4	1.4	2.8	1.46	5
Whole	90.0	0	4.46(H)	6.20	97.3	60.0	25.3	7.0	-	7.6[c]	2.90	6
Whole	82.0	7	5.23(H)	6.30	101.3	65.9	14.2	12.8	-	7.2[c]	5.10	6

[a] Feeding frequency indicated by: Al- ad libitum, D- once daily, H- hourly.

[b] Ac- acetate, Bu- butyrate, IV- isovalerate, Pr- propionate, V- valerate.

[c] Isobutyrate + isovalerate + valerate.

[d] Reference

1. Vance et al. (1970)
2. White et al. (1972)
3. Vance et al. (1972)
4. Rounds (1973)
5. White et al. (1975b)
6. Cole et al. (1976a)

various ruminant sub-systems and, further, the subsequent integration of these sub-systems into animal and production system models should provide a new generation of qualitative and quantitative tools with which to study and evaluate ruminant production (Baldwin et al., 1977).

The remainder of this manuscript will deal with the description and evaluation of experiments designed to study VFA production and metabolism in the rumen of steers consuming high-concentrate whole or ground corn diets. In discussion of the results obtained, frequent reference will be made to works presented in this review.

CHAPTER IV

EXPERIMENTAL PROCEDURES

Introduction

Twelve separate infusions of a labeled volatile fatty acid were conducted using three Hereford steers. These steers were fitted with permanent rumen cannulae (2.5 cm I.D.) and fed at hourly intervals either a whole or ground shelled corn high-concentrate ration. The experiments, as illustrated in Table VIII, were conducted in two phases. During Phase I, Animal 608 was fed the ground corn ration and on three separate occasions was infused intraruminally with either 1-^{14}C-acetate, 2-^{14}C-propionate or 1-^{14}C-butyrate. Also during Phase I, Animal 422 was fed the whole corn ration and was infused in a similar fashion.

Phase II was originally planned to be a reversal of the animal-ration combinations used in Phase I, thus being a switchback design. However, before initiating Phase II infusions with Animal 422, a laboratory accident, unrelated to the treatments, deemed it necessary to remove this animal from the experiment. Consequently, Animal 612 was adapted to the ground corn ration and completed the second half of Phase II.

TABLE VIII

EXPERIMENTAL DESIGN SHOWING ANIMALS, RATIONS AND INFUSION DATES

	Ground Corn		Whole Corn	
	Phase I	Phase II	Phase I	Phase II
Animal	608	612	422	608
Acid Infused				
Ac	9-16-71	2-09-72	9-17-71	10-24-71
Pr	10-02-71	1-22-72	10-01-71	11-23-71
Bu	9-23-71	1-29-72	9-24-71	11-11-71

Animals and Facilities

Previous history of these animals has been described by Rounds (1973). Animals were housed in tubular-frame metabolism stalls[1] located in the basement of Veterinary Medicine (South). The metabolism stall measured 0.9 m wide, 1.9 m long and 1.3 m high. The floor of the stall was 0.53 m above the floor. Occasionally the animals were taken out of the stalls and exercised. Air conditioning and heating systems provided adequate ventilation and odor control and maintained a relatively constant temperature of 21°C. Animals were adapted to the metabolism stalls from previous experiments (Rounds, 1973). In addition, the animals previously had been consuming either the whole or ground corn ration. Adaptation to a change in corn processing

was considered complete in two weeks.

Animals were weighed before and after each infusion period as indicated in Table IX.

TABLE IX

ANIMAL WEIGHTS BEFORE AND AFTER INFUSION PERIODS

Phase	Date	Ration	Animal	Kg	Mean Period Wt. Kg	Mean Period Wt. $Kg^{.75}$
I-1	9-15-71	GC	608	350	357	82.1
	10-03-71	GC	608	363		
I-2	9-15-71	WC	422	371	379	86.0
	10-03-71	WC	422	388		
II-1	10-22-71	WC	608	381	393	88.3
	11-24-71	WC	608	405		
II-2	1-20-72	GC	612	446	454	98.3
	2-10-72	GC	612	462		

Rations and Feeding Regime

Ration Composition

Rations were identical to those fed by Rounds (1973). Ration composition is presented in Table X. Corn ground through an 8.78 mm screen in a hammermill was used for the ground corn ration. The supplement and alfalfa were pelleted separately through a 9.5 mm die.

TABLE X

COMPOSITION OF RATIONS

Ingredient	IRN[a]	%, DM Basis	
		WC	GC
Corn, dent yellow, grain, (4)[b]	4-02-935	84.0 -	- 84.0
Cotton, seed hulls, (1)	1-01-599	7.0	7.0
Alfalfa, aerial pt., dehy. grnd, (1)	1-00-025	3.0	3.0
Supplement		6.0	6.0
Soybean, seed, solv-extd grnd, mx 7 fbr, (5)	5-04-604		3.28
Urea, mn 45% nitrogen, (5)	5-05-070		0.69
Calcium carbonate, commercial, mn 38% Ca, (6)	6-01-069		0.54
Calcium phosphate, dibasic, commercial, (6)	6-01-080		0.54
Potassium chloride, KCl, CP, (6)	6-03-756		0.33
Salt	...		0.33
Wheat, flour by-prod, mx 9.5 fbr, (4)	4-05-205		0.18
Aurofac-10[c]	...		0.06
Stilbestrol[d]	...		0.025
Trace mineral mix (Stillwater mill)	...		0.015
Vitamin A palmitate, commercial (7)[e]	7-05-143		0.01

[a]International Reference Number, Atlas of Nutritional Data on United States and Canadian Feeds. 1971. National Academy of Sciences, Washington, D.C.

[b]Corn - ground through an 8.78 mm screen.

[c]Provides 13.2 mg Chlortetracycline per kilogram of feed.

[d]Elanco (4.4 mg/g); provides 2.64 mg diethylstilbestrol per kilogram of feed.

[e]30,000 IU per gram; provides 3,000 IU per kilogram of feed.

Feed Analysis

Samples of feed were collected during each infusion experiment. Samples were ground through a 1 mm screen in a Wiley mill and stored in plastic bottles at room temperature. These samples were analyzed for dry matter, ash and crude protein (A.O.A.C., 1965). The mean chemical composition of the rations is shown in Table XI. Values for gross energy were obtained from Rounds (1973).

TABLE XI

CHEMICAL COMPOSITION OF RATIONS[a]

Item	Units	Whole Corn	Ground Corn
Dry Matter	%	89.14	87.37
Ash	%	2.89	2.66
Crude Protein	%	12.83	12.77
Gross Energy[b]	MJ/kg	18.60	18.81

[a]On a dry matter basis.

[b]Data from Rounds (1973) as determined by bomb calorimetry.

Particle size of the ground corn was determined by the method reported by Ensor et al. (1970). The particle size distribution of the ground corn is presented in Table XII. Whole corn grain was

found to be completely retained on the 4000 micron screen.

TABLE XII

PHYSICAL CHARACTERISTICS OF GROUND CORN GRAIN

Item	% Retained on Screen
Sieve diameter (microns)	
4000	-
2000	0.6
1000	25.0
500	32.9
250	18.1
125	9.0
pan	14.4
Geometric mean diameter diameter[a] (microns)	477
Geometric standard deviation[a]	2.68

[a]Procedure described by Ensor et al. (1970).

Feeding System

To achieve steady-state conditions in the rumen, an individual feeding system was used which dispensed a given amount of feed hourly. The system consisted of an Eriez Vibratory Feeder Model FBV-212[2]. This system employs a 0.043 m^3 feed hopper with an attached magnetic

vibrator. The hopper feeds through an 8 cm diameter chute into a 50 cm long, V-shaped slide trough which also is equipped with a magnetic vibrator attached to the underside of the trough. Both vibrators are wired to separate rheostat controls for controlling the frequency and intensity of vibration. The proximity of the hopper spout to the slide trough can be adjusted by means of an outside sleeve over the spout. This adjustment allows for regulation of the amount of feed flowing from the hopper into the slide trough. Additionally, the rate of feed flow can be controlled by the modulation of the two vibrators. The entire feeder unit is controlled through a programmed time control mechanism[3]. This time control allows for selection of time intervals of signals within the range of one to twenty-four per 24 hour period. In addition, the duration of the signal can be varied from 5 to 60 seconds.

In this study, the position of the sleeve over the spout and the intensity of vibrations were adjusted so that relatively free flow of the ration from the hopper to the slide chute could be accomplished. It was found that the quantity of feed delivered per feeding could conveniently be controlled simply by regulating the time duration of the signal from the timer. Using this procedure, each feeder was calibrated with each ration according to feed delivery rate and signal duration. Calibration settings were checked periodically. A daily record of actual feed intake was obtained by weighing back each morning the amount of feed not dispensed.

Experiments in this laboratory (Hinman and Johnson, 1974; Kropp, 1975) using these feeders where animals were fed at hourly intervals have demonstrated reduced diurnal variations in rumen fermentation

parameters (pH, NH_3-N concentration) and digesta flow into the lower tract.

Steers adapted quickly to this type of feeding system and were often observed anticipating each hourly feeding. Usually each feeding was consumed within 5 minutes; however, at night it was noted that occasionally several feedings would accumulate before eating. Therefore, in an effort to minimize any effect of eratic feed consumption at night, experiments were not initiated until mid-morning (usually 1100 hours). The animals were offered feed and water ad libitum; however, water was unavailable during each infusion experiment.

Tracer Administration

Radioactive VFAs

The VFA isotopes, 1-^{14}C-acetate, 1-^{14}C-butyrate and 2-^{14}C-propionate, were obtained commercially from ICN Laboratories[4]. The specific activity of the ^{14}C acetate, butyrate and propionate was 56 mCi/mmole, 25 mCi/mmole and 3 mCi/mmole, respectively. The radioactive purity of each ^{14}C-compound was determined by thin-layer chromatography (TLC)/autoradiochromatography procedures as described by Schwarz/Mann[5] and discussed below.

Samples of each batch of isotope were applied to pre-coated analytical TLC plates[6]. Before spotting, plates were pre-developed in the solvent system subsequently used. Solvent systems were chosen which would resolve at least acetic, propionic and butyric acids. Hamilton[7] syringes (10 µl) were used to apply samples to the plates. Three dilutions (100, 10 and 1%) of each isotope solution were spotted

per plate. Two plates per isotope were prepared. After sample application, the plates were developed. Duplicate plates were developed in different solvent systems. The solvents systems, v/v, used were as follows:

(a) Ethanol:Ammonium Hydroxide:Water (100:16:12)

(b) 2-propanol:Ammonium Hydroxide (3:1)

Position and relative intensity of the migrated spots and origins was detected by exposing the surface of the plates to X-ray film[8] for approximately 12 hours. The film was developed and the relative intensity of the migrated spot and origin were compared. Also, the migrated spots and the origins were scraped into scintillation vials containing 10 ml of Brays (Bray, 1960) scintillation solvent (Table XIII) and counted in a liquid scintillation spectrometer[9] with quench correction provided by automatic external standardization. Recovery of total radioactivity was generally 98-103%.

In addition, radiochemical purity of each ^{14}C-compound was checked by a buffered silica gel, chloroform-butanol liquid chromatography system (to be described later).

Estimates of radiochemical purity obtained by these two techniques were quite comparable. It was found that the radioactive purity of ^{14}C-acetate, -butyrate and -propionate was 101, 95.9 and 95.1%, respectively. The identity of the radioactive impurity is unknown. However, it did not appear to be another VFA since no unaccountable radioactivity was found to occur in the 'unlabeled' VFA peaks eluted from the silicic acid columns. The impurity appeared to remain in the top portion of the column gel and was not eluted with a 65:35 (v/v) n-butanol:chloroform solvent. With the TLC system, the impurity

appeared to remain at the origin. Tracer concentration in propionate and butyrate infusion solutions was adjusted for the contaminant.

TABLE XIII

FORMULA FOR 'BRAYS' SCINTILLATION FLUID[a,b]

Item	Quantity/liter
PPO, (g)[c]	5.0
M_2-POPOP, (g)[c]	0.5
Naphthalene, (g)[d]	60.0
Methanol, (ml)[e]	100.0
Ethylene glycol, (ml)[e]	20.0
1,4-Dioxane[d]	to volume

[a]Bray (1965).
[b]All reagents are scintillation grade.
[c]Packard Instrument Company.
[d]Mallinckrodt Chemical Works.
[e]Matheson, Coleman and Bell.

Infusion Solutions

Radioactive VFAs were diluted with glass distilled water and stored in tightly stoppered containers at 4°C. Dilutions were calculated according to the known delivery rate of the infusion pump and desired rate of tracer infusion. The rate of tracer infused to each VFA compartment was estimated from the relationship

$$\frac{u_i}{f_i} = a_i \tag{4.1}$$

where values for f_i (turnover rate) were extrapolated from available literature data and a_i (specific activity) was chosen to be of an order of magnitude that could be precisely determined in the laboratory. Since a priming dose of tracer initiated each infusion, the amount of priming dose to be injected was calculated from the equation

$$k_i = \frac{u_i}{D_i} = \frac{f_i}{Q_i} \tag{4.2}$$

where D_i represents the priming dose and f_i and Q_i were estimated from literature values.

Aliquots of the infusion solution used in each experiment were taken and counted in Bray's scintillation solution with a liquid scintillation spectrometer[9].

The priming dose and rate of isotope infusion (corrected for radiochemical purity) for each experiment is presented in Table XIV.

Polyethylene Glycol Solution

The rumen liquid volume and out-flow to the omasum were estimated after an intraruminal injection of 50 g polyethylene glycol (PEG)[10], average molecular weight 3000 - 3700. A solution containing 50 g of PEG in 200 ml distilled water was prepared just prior to each experiment.

TABLE XIV

INFUSION RATES OF ^{14}C-LABELED VFAS

Animal	VFA Infused[a]	Ration	D[b] (μCi)	u[c] (μCi/min)
608	Ac	GC	218.0	1.90
608	Pr	GC	102.0	1.11
608	Bu	GC	56.5	0.469
422	Ac	WC	167.0	1.46
422	Pr	WC	104.0	1.11
422	Bu	WC	56.5	0.461
608	Ac	WC	176.0	1.46
608	Pr	WC	97.2	1.06
608	Bu	WC	56.5	0.469
612	Ac	GC	213.0	1.89
612	Pr	GC	94.1	0.986
612	Bu	GC	56.3	0.393

[a]Ac: 1-^{14}C-acetate, Pr: 2-^{14}C-propionate, Bu: 1-^{14}C-butyrate.

[b]Priming dose.

[c]Infusion rate.

Protocol of Tracer Administration

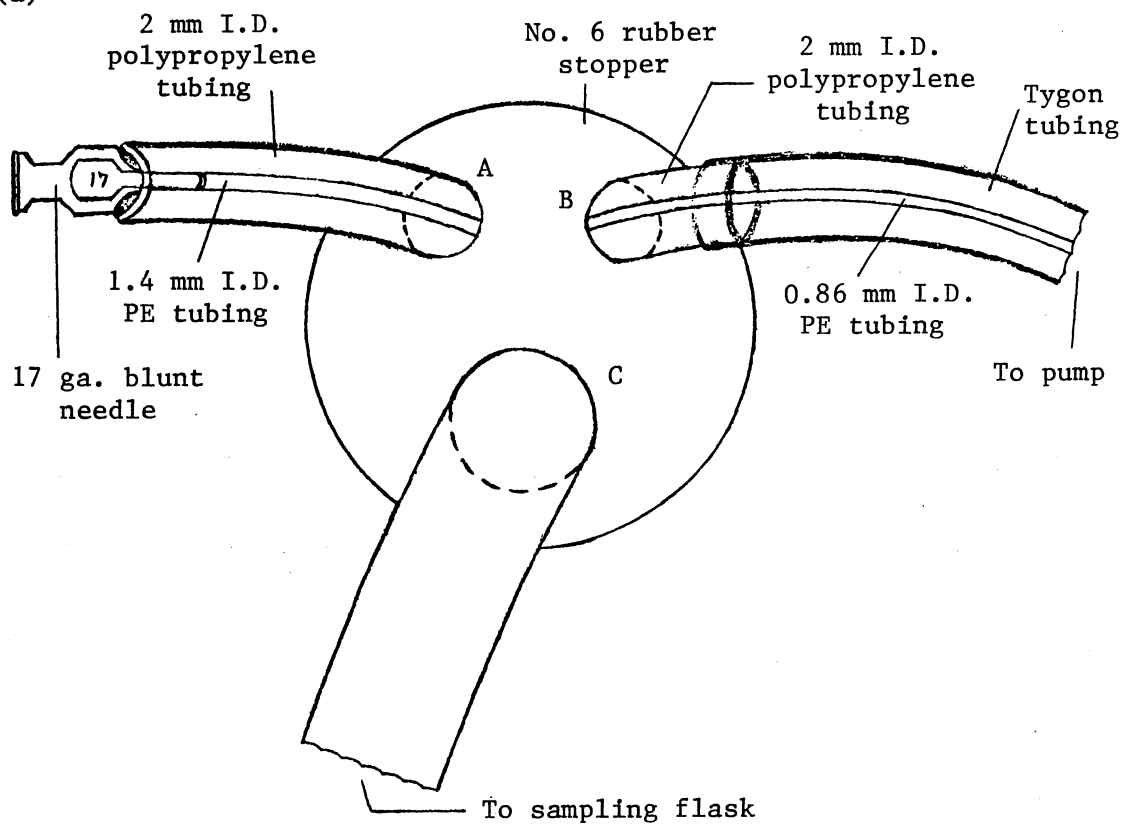
Experiments were begun approximately mid-way between the hourly feedings. Infusions were initiated with the injection of the ^{14}C-VFA priming dose into the rumen. The time was noted and all subsequent time measurements were made relative to this initial time. Immediately following the priming dose injection, a continuous infusion of the same ^{14}C-VFA was commenced and continued for 6 hours. The starting of the infusion pump was followed by the intraruminal injection of the PEG solution.

A separate port in the cannulae bung (Figure 15), not associated with the infusion port, was used to inject the ^{14}C-VFA and PEG doses. This port consisted of a section of stiff, polypropylene tubing (2 mm I.D.) approximately 20 cm long which completely passed through the bung and protruded about 5 cm outside the cannulae. The outside end of this tubing was fitted with a blunt 17 gauge hypodermic needle to which was attached a 21 cm section of polyethylene (PE) tubing[11] (1.4 mm I.D.). This PE tubing was routed inside the polypropylene tubing so that the distal end of the PE tubing protruded approximately 1 cm past the end of the outer sleeve. This arrangement made it possible to disperse the tracer solutions more uniformily throughout the rumen by manipulating the external portion of the polypropylene sleeve as the tracer was being injected. This injection port was rinsed with approximately 100 ml water after each tracer injection.

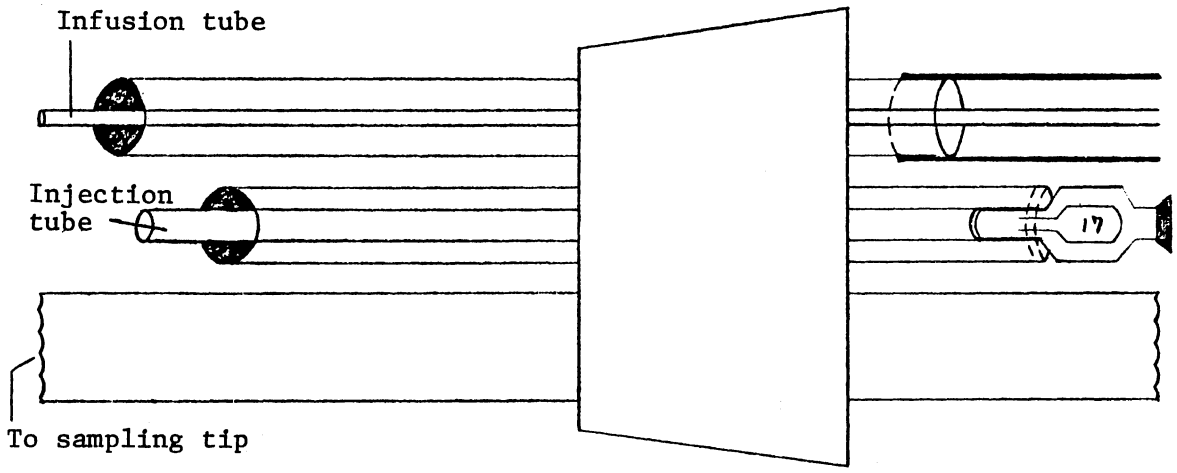
Just prior to each experiment the radioactive solution to be infused was drawn into a disposable plastic syringe and mounted in a Harvard syringe pump[12] which had been previously calibrated. The

Figure 15. Diagrammatic Sketches of the Cannulae Bung and Input-Output Ports. (a) Outside view of the bung and various ports, A- injection port, B- infusion port, C- sampling port; (b) Side view of bung; (c) Crossectional, caudal view of the steer and the relationship of the sampling and tracer administration tubes to the rumen.

(a)


2 mm I.D.
polypropylene
tubing
No. 6 rubber
stopper
2 mm I.D.
polypropylene
tubing
Tygon
tubing
17
A
B
1.4 mm I.D.
PE tubing
0.86 mm I.D.
PE tubing
17 ga. blunt
needle
C
To pump
To sampling flask

(b)


Infusion tube
Injection
tube
17
To sampling tip

(c)

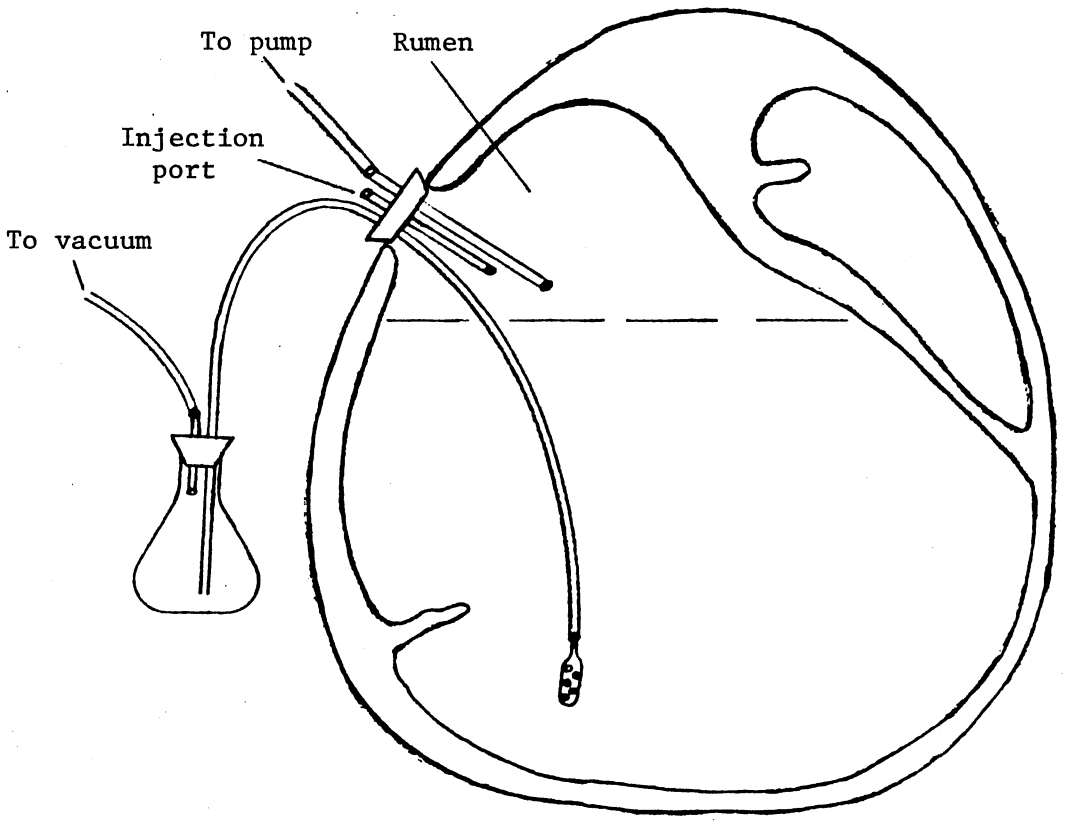


Figure 15. (Continued)

output of the syringe was piped through a length of PE tubing (0.86 mm I.D.) which terminated inside the rumen of the steer. The PE tubing was routed through the cannulae bung and into a stiff, polypropylene sleeve (2 mm I.D.) which was embedded in the rubber bung on one end and protruded approximately 25 cm into the air space of the rumen. The end of the PE tubing was positioned approximately 1 cm distal to the end of the sleeve. This arrangement maintained a relatively constant position of the point of infusion.

Sampling

Rumen Fluid

Samples of rumen contents were collected prior to tracer administration and at hourly intervals for nine hours following the ^{14}C-VFA dose. Samples were obtained by suction through a third port in the cannulae bung. This port consisted of a 70 cm length of stiff, polypropylene tubing (5 mm I.D.) which passed completely through the cannulae bung. Approximately 40 cm of tubing protruded through the bung and was suspended in the rumen. To the rumen end of this tube was attached a metal sampling tip which consisted of a stainless steel capsule perforated with numerous 2 mm diameter holes. According to this configuration, the sampling tip was situated such that samples were taken from the lower portion of the ventral sac. The external portion of the sampling tube was allowed to hang free until a sample was to be taken. A sketch of the cannulae bung and the spatial relationship of the three input-output ports in relation to the rumen are presented in Figure 15.

When it was time to sample, the sampling tube was attached to a suction flask. Approximately 50 ml of fluid was collected and discarded. This was followed by the collection of approximately 200 ml of fluid. As quickly as possible the fluid was strained through four layers of cheesecloth and 100 ml of this filtrate was added to a flask containing 0.5 ml of a saturated mercuric chloride solution. The pH was measured on the remaining fluid after which a 30 ml sample was saved for PEG analysis. Immediately following the pH determination, samples were poured into appropriately labeled polypropylene containers and stored at -25°C. All glass and plastic ware was thoroughly washed prior to the next sampling period.

Blood

During the morning prior to an experiment, the steer was fitted with an indwelling catheter in the left jugular vein. The catheter consisted of a suitable length of PE tubing (1.4 mm I.D.) which extended into the vein approximately 3.5 cm past the point of entry. The length of tubing immediately outside the animal was held in place by tape and skin sutures.

A 50 ml sample of blood was taken as close as possible to the time of rumen sampling. The blood was drawn into a syringe containing 5 ml of a 1 mg/ml heparin solution. After the blood sample has been obtained, the catheter was flushed with 25 ml of a 2% sodium citrate solution. The sample of blood was immediately cooled to 4°C and centrifuged at 12,000 x g for 10 minutes. The plasma was decanted into plastic bottles and stored at -25°C.

Rumen Fluid Analysis

The rumin fluid containing mercuric chloride was used for all determinations of ^{14}C-VFA specific activity and VFA concentration. After samples were thawed and shaken thoroughly, approximately 25 g of fluid was centrifuged at 48,000 x g for 20 minutes. The resulting supernatant was relatively clear but had a slight straw color. This supernatant was used in all subsequent ^{14}C and VFA analysis.

The samples of rumen fluid not treated with mercuric chloride were used in the determination of PEG.

Determination of VFA Specific Activity

A liquid-liquid partition chromatography system (LLC) using buffered silicic acid columns was developed. This made it possible to separate and collect, on a quantitative scale, the ^{14}C-VFAs present in the rumen fluid samples. Silica gel column techniques and procedures used were similar to those employed by Moyle *et al*. (1948).

Silicic Acid Columns

Glass columns[13] (25 mm I.D. x 250 mm long) fitted with removable polyethylene filter discs (porosity of 40 - 60 microns) and adjustable flow teflon (TFE) stopcocks were used. Column effluent was conveyed to a Technicon proportioning pump[14] through 1.06 mm I.D. TFE tubing.

Silicic acid[15] (100 mesh, reagent grade) was prepared for use by removing the fine particles which tended to impede solvent flow through the column. Approximately 350 g of the silicic acid was suspended in 1 liter of distilled water and was allowed to settle for

3 minutes. The supernatant was discarded and the settled particles were transferred to a pie plate and dried in a 105°C oven.

Ten grams of prepared silicic acid were intimately mixed with 6 ml of a phosphate buffer[16], pH 7.35 in a medium-sized mortar. This mass was slurried in approximately 70 ml of chloroform[17] and then poured into a closed column. Additional chloroform was used to rinse residues of the silicic acid mixture from the mortar and sides of the column. A glass stirring rod was employed to mix the contents of the column. After mixing, the stopcock was opened to allow the chloroform to percolate through the column at a rate of 1 - 2 ml per minute. Packing of the gel was facilitated by gentle tapping of the column side. Usually 30-45 minutes were required to achieve proper column development as characterized by formation of a firm gel surface as well as a stationary gel height. After the column was developed, the stopcock was closed and the space above the gel was filled with 50 ml chloroform.

A 3 ml volume of rumen fluid (48,000 x g supernatant) was titurated with 5 g of silicic acid in a medium-sized mortar. This free-flowing powder was carefully added to the solvent space on top of the column. The sides of the column were washed with chloroform and then the solvent layer was gently stirred with a glass rod. Stirring was continued until all particles of the sample were dispersed and wetted with chloroform while care was taken not to disturb the packed gel. After stirring was complete, the stopcock was opened and solvent was allowed to flow through the column at a rate of 1-2 ml per minute. Again, packing was aided by gentle tapping and was considered complete (usually in about 20 minutes) when gel height remained stationary. As

soon as the chloroform in the head space drained into the gel, the stopcock was closed and 2 ml of a 1% (v/v) butanol[18]-chloroform mixture was placed on top of the column. The top end of the columns were then connected to the gradient elution system.

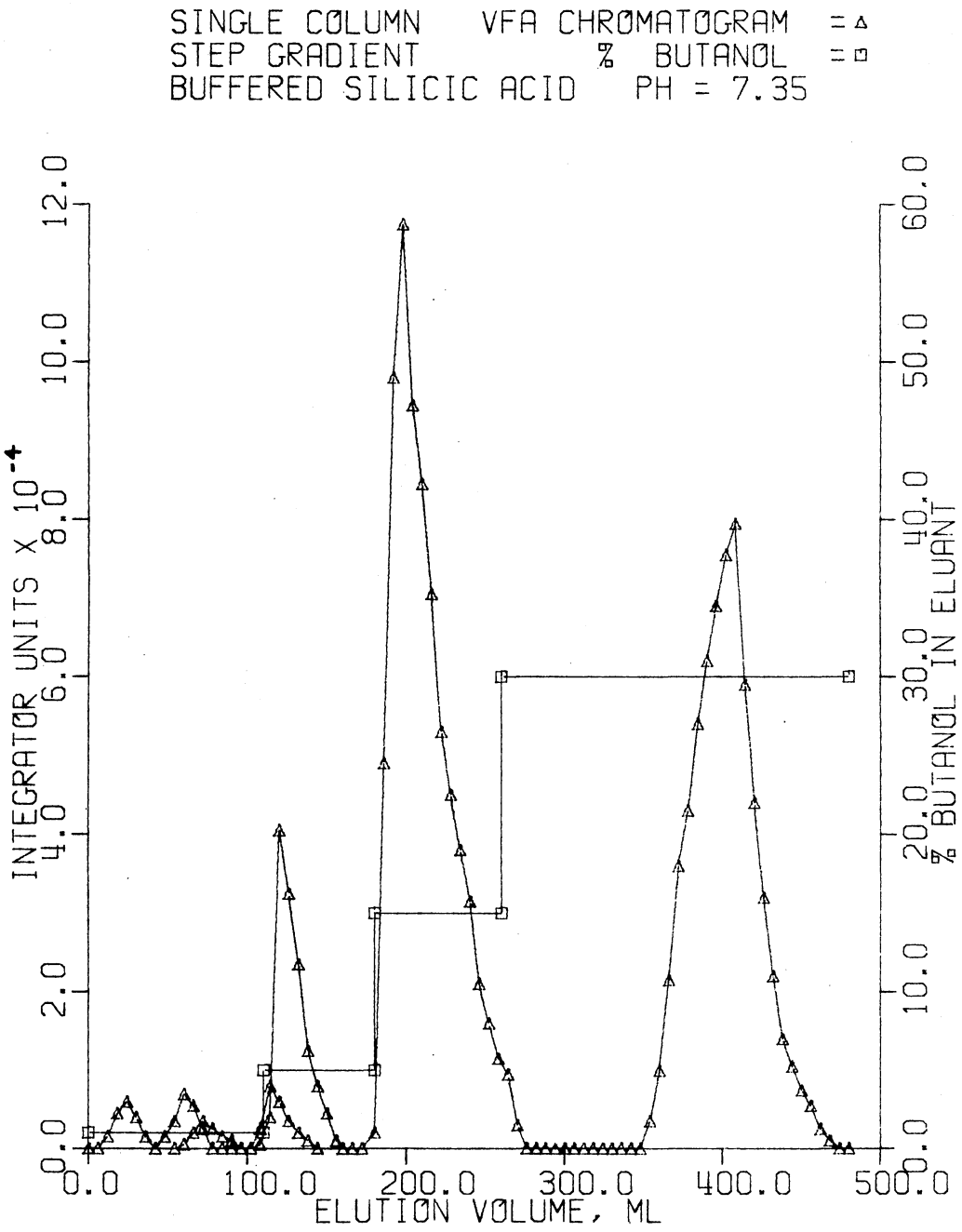
Gradient Elution System

Initially a step gradient (Figure 16) was used to elute the VFAs from the silicic acid columns. Although this gradient usually provided an acceptable degree of acid separation (Figure 16), it was unsatisfactory because (1) columns needed to be monitored closely so that the next solvent step could be added before the column ran dry, (2) resolution between the C_4 and C_5 acids was not always complete and (3) tailing of peaks was sometimes excessive. Therefore, a continuous non-linear gradient system was devised to alleviate these problems by automatically and gradually increasing the concentration of n-butanol in the elution phase.

Most of the techniques available (Peterson and Sober, 1959; Castellana and Kelly, 1973) for generating non-linear gradients involve (1) pumping and switching systems which are either complex and expensive or difficult to operate, or (2) specifically shaped apparatus which limit the versatility of the system. Consequently, a continuous gradient system was developed which did not depend on the shape of the mixing chambers and the rate of flow of eluent through the columns regulated the flow of solvent through the system. It was further desired that the gradient system could be easily adapted to feed several columns simultaneously.

Basically, the system consisted of three serially connected

Figure 16. Fractionation of VFAs from Rumen Fluid (06-608-Ac-GC) on a Buffered (pH 7.35) Silicic Acid Column Using a Chloroform-Butanol Step Gradient. VFAs were detected by gas chromatography (see text).

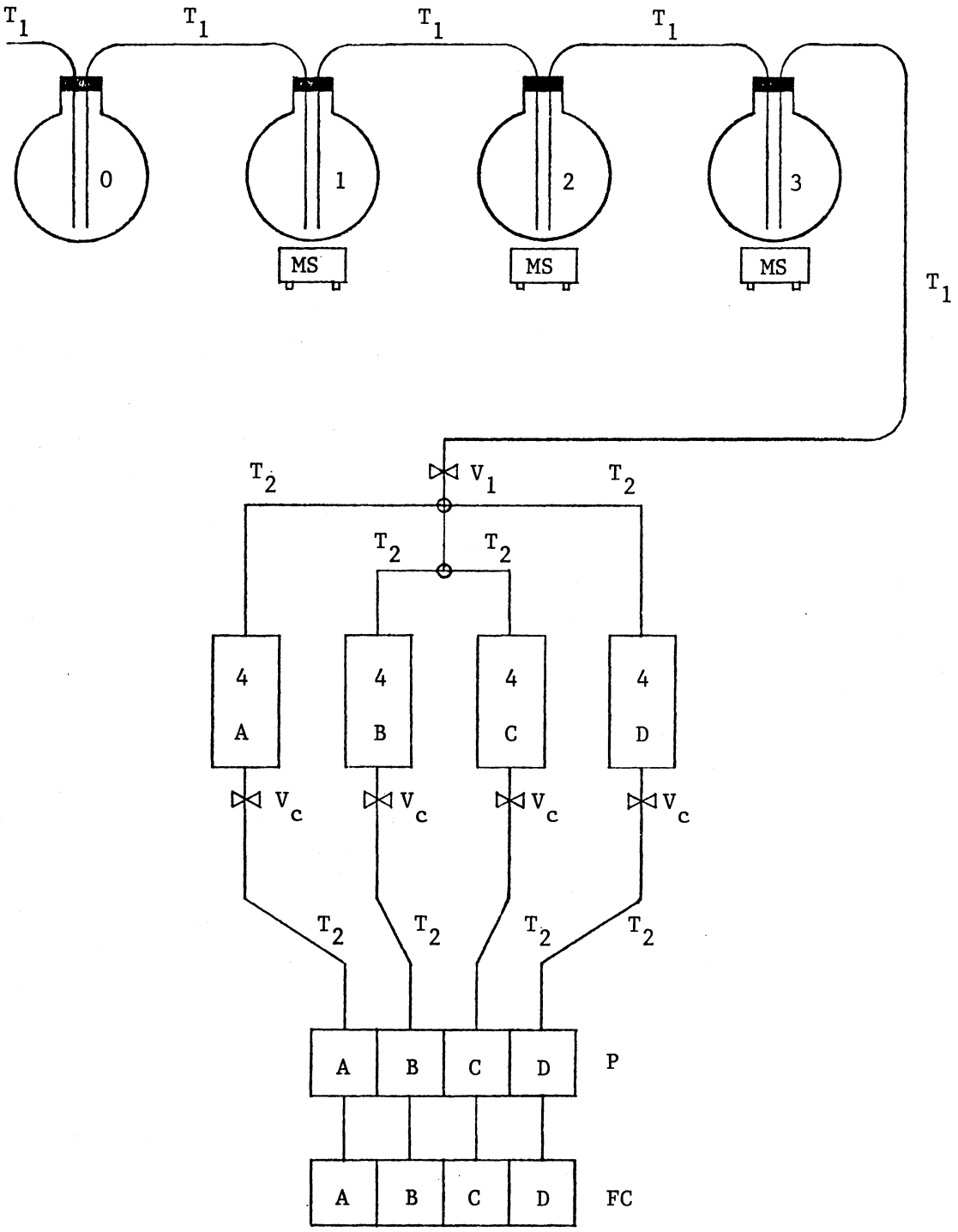

SINGLE COLUMN VFA CHROMATOGRAM = △
STEP GRADIENT % BUTANOL = □
BUFFERED SILICIC ACID PH = 7.35
INTEGRATOR UNITS X 10⁻⁴
0.0
2.0
4.0
6.0
8.0
10.0
12.0
% BUTANOL IN ELUANT
0.0
10.0
20.0
30.0
40.0
50.0
60.0
0.0
100.0
200.0
300.0
400.0
500.0
ELUTION VOLUME, ML

mixing chambers and a terminal reservoir of high eluting strength solvent (n-butanol). A schematic diagram of the entire chromatographic system is presented in Figure 17. Solvent reservoirs consisted of 500 ml round bottom flasks and each of the three mixing chambers were constantly stirred by a magnetic stirring bar apparatus. Solvent removed from the system was replaced with air which could enter only through reservoir 0.

Initial concentrations of n-butanol in water-washed chloroform and compartmental volumes were determined by trial and error. System performance was evaluated on the basis of the apparent resolution between the C_5 and C_4 acids and the volume required to elute acetic acid. These trial and error experiments were aided by the use of a compartment model describing the kinetics of a series of consecutive irreversible first order reactions in which the order of the system is reduced by one when the supply of solvent in the terminal compartment is exhausted. The mathematics of similar systems have been described by Bailey et al. (1974). A mathematical model was constructed and simulated on a digital computer (IBM 370/158) using the Continuous System Modeling Program (CSMP) developed by IBM (1969). Using the model, initial butanol concentrations and compartment volumes were adjusted until the simulated concentration of butanol in the column eluant (compartment 3) corresponded to the steps of increasing butanol concentration in the discontinuous gradient shown previously in Figure 16.

Using the initial conditions determined from the simulation experiments, actual chromatograms were obtained and, based on the column performance criteria already mentioned, these initial conditions

Figure 17. Schematic Diagram of the Continuous Butanol-Chloroform Gradient System. Notation in the figure corresponds to: 0- butanol reservoir; 1,2,3- mixing chambers; MS- magnetic stirring units; 4(A-D)- silicic acid columns; FC(A-D)- respective rows in the fraction collector; P- peristaltic pump; T_1- TFE tubing (2.0 mm I.D.); T_2 - TFE tubing (1.06 mm I.D.); V_1- inlet control valve; V_C(A-D)- column flow control valve.


T1
T1
T1
T1
0
1
2
3
MS
MS
MS
T1
T2
V1
T2
T2
T2
4
A
4
B
4
C
4
D
Vc
Vc
Vc
Vc
T2
T2
T2
T2
A
B
C
D
P
A
B
C
D
FC

were adjusted so as to minimize the time required to elute acetic acid while maintaining a satisfactory level of resolution between the C_4 and C_5 acids. During these preliminary experiments it was noted that each transition of the system from order n to order n-1 was accompanied by a readjustment of the hydrostatic equilibrium within the system to a new steady-state level. This period usually lasted 1-3 minutes and involved primarily an increase in the volume of the solvent head on the columns and a concomitant increase in throughput of solvent in the remaining compartments of the system. These system dynamics were generally quite reproducible and those which could be easily quantitated were subsequently incorporated into the simulation model. System initial conditions used for all sample separations are listed in Table XV. The computer model is given in Table XVI.

TABLE XV

GRADIENT SYSTEM INITIAL CONDITIONS[a]

Compartment	Solvent volume (ml)	Concentration of butanol (% v/v)
0	425	100
1	480	4
2	440	5
3	480	1
4[b]	16	1

[a]Values are for a four column system.

[b]Represents the lumping of four columns into one compartment.

TABLE XVI

CSMP MODEL OF THE NON-LINEAR CONTINUOUS
BUTANOL-CHLOROFORM GRADIENT SYSTEM

```
TITLE NONLINEAR BUOH/CHCL3 GRADIENT FOR SILICIC ACID
TITLE LIQUID-LIQUID PARTITION COLUMN CHROMATOGRAPHY
TITLE IRREVERSIBLE 5 COMPARTMENT SYSTEM, 4 COLUMNS
TITLE OPERATIONAL
RENAME TIME=T
FIXED K1,K2,K3,K4
INITIAL
NOSORT
PARAMETER Y0=100.0, Y1=4.0,Y2=5.0, Y3=1.0, Y4=0.0, Y5=0.0
PARAMETER V0A=425.0, V1A=480.0, V2A=440.0, V3A=480.0, V4A=16.0, V5A=0.0
PARAMETER FR=2.0, CC = 0.008098
      CC0=Y0*CC
      CC1=Y1*CC
      CC2=Y2*CC
      CC3=Y3*CC
      CC4=Y4*CC
      CC5=Y5*CC
      K1=0
      K2=0
      K3=0
      K4=0
      FN = 1.0
      QFP=0.0
      VP=0.0
      A = 0.0
      B = 0.0
      C = 0.0
      D = 0.0
      Q0A=Y0*V0A*CC
      Q1A=Y1*V1A*CC
      Q2A=Y2*V2A*CC
      Q3A=Y3*V3A*CC
      Q4A=Y4*V4A*CC
      Q5A = Y5*V5A*CC
      T0 = 0.0
      T1 = 0.0
      T2 = 0.0
      T3 = 0.0
      T4 = 1000.0
FUNCTION FS = 0.0,30.5,15.0,30.5,90.0,29.5,133.0,28.5,...
445.0,29.0,547.0,29.5,738.0,28.6
DYNAMIC
NOSORT
      T0 = 0.0
      IF(T.EQ.0.0) T0=1.0
      X1 = PULSE(2.0,T0)
      X2 = PULSE(1.0, T1)
      X3 = PULSE(2.0, T2)
      X4 = PULSE(2.0, T3)
      F = FR + X1*18.0 + X2*6.0 + X3*13.0 + X4*8.0
      F0 = FR*(1.0 - X1)
      DQ0DT = -F*CC0
      DQ1DT = F*CC0 - F*CC1
      DQ2DT = F*CC1 - F*CC2
      DQ3DT = F*CC2 - F*CC3
      DQ4DT = F*CC3 - F0*CC4
      DQ5DT = F0*CC4
```

TABLE XVI (Continued)

```
      Q0 = INTGRL(Q0A, DQ0DT)
      Q1 = INTGRL(Q1A, DQ1DT)
      Q2 = INTGRL(Q2A, DQ2DT)
      Q3 = INTGRL(Q3A, DQ3DT)
      Q4 = INTGRL(Q4A, DQ4DT)
      Q5 = INTGRL(Q5A, DQ5DT)
      F1 = -F
      V0 = INTGRL(V0A,F1)
      F2 = -(F*A)
      V1 = INTGRL(V1A,F2)
      F3 = -(F*B)
      V2 = INTGRL(V2A,F3)
      F4 = -(F*C)
      V3 = INTGRL(V3A,F4)
      E = 1.0 - D
      F5 = F*E - F0
      V4 = INTGRL(V4A,F5)
      F6 = F0
      V5 = INTGRL(V5A,F6)
      V = F0*T - VP
      VF = AFGEN( FS, T)
      QF = Q5  - QFP
      IF( VF - V ) 20,20,21
   21 CONTINUE
      IF(V0) 2,2,100
  100 C0 = Q0/(V0*CC)
      CC0 = C0*CC
    1 CONTINUE
      IF(V1) 3,3,400
  400 C1 = Q1/(V1*CC)
      CC1 = C1*CC
    4 CONTINUE
      IF(V2) 5,5,600
  600 C2 = Q2/(V2*CC)
      CC2 = C2*CC
    6 CONTINUE
      IF(V3) 7,7,800
  800 C3 = Q3/(V3*CC)
      CC3 = C3*CC
    8 CONTINUE
      IF(V4) 9,9,900
  900 C4 = Q4/(V4*CC)
      CC4 = C4*CC
  901 CONTINUE
      IF(V5) 99,99,990
  990 C5 = Q5/(V5*CC)
      CC5 = C5*CC
  991 CONTINUE
FINISH V4 = 0.0
      GO TO 10
   20 QFP = QF + QFP
      VP = V + VP
      CF = QF/(VF*CC)
      FN = FN + 1.0
      GO TO 21
    2 C0 = 0.0
      CC0 = 0.0
      Q0 = 0.0
      V0 = 0.0
      T1 = 0.0
      IF(K1.EQ.0) T1=T
      K1 = K1 + 1
      A = STEP(T1)
      GO TO 1
```

TABLE XVI (Continued)

```
    3 C1 = 0.0
      CC1 = 0.0
      Q1 = 0.0
      V1 = 0.0
      T2 = 0.0
      IF(K2.LE.1) T2=T
      K2 = K2 + 1
      B = STEP(T2)
      GO TO 4
    5 C2 = 0.0
      CC2 = 0.0
      Q2 = 0.0
      V2 = 0.0
      T3 = 0.0
      IF(K3.LE.1) T3=T
      K3 = K3 + 1
      C = STEP(T3)
      GO TO 6
    7 C3 = 0.0
      CC3 = 0.0
      Q3 = 0.0
      V3 = 0.0
      IF(K4.EQ.0) T4=T
      K4 = K4 + 1
      D = STEP(T4)
      GO TO 8
    9 C4 = 0.0
      CC4 = 0.0
      Q4 = 0.0
      V4 = 0.0
      GO TO 901
   99 C5 = 0.0
      CC5 = 0.0
      Q5 = 0.0
      V5 = 0.0
      GO TO 991
   10 CONTINUE
PRINT FN,F,QO,CCO,CF,FO,Q1,CC1,QF,DQODT,Q2,CC2,VF,DQ1DT,...
Q3,CC3,V,DQ2DT,Q4,CC4,VP,DQ3DT,Q5,CC5,QFP,DQ4DT,T1,CO,VO,...
DQ5DT,T2,C1,V1,X1,T3,C2,V2,X2,T4,C3,V3,X3,A,C4,V4,X4,B,...
C5,V5
PRTPLT Q4(V5, DQ4DT, C4), CF(VF, QF)
PRTPLT CF(FN,QF,VF), Q5(V5,DQ5DT,C5), C3(V3,DQ3DT,Q3)
METHOD RKSFX
TIMER DELT=1.0, FINTIM=10.0, OUTDEL=1.0, DELMIN=1.0E-4
END
STOP
ENDJOB
```

Figure 18 illustrates the actual concentration of n-butanol in the column fractions as compared with the calculated concentrations obtained from the simulation model. Agreement between the model and the actual data is acceptable. The model overestimates the butanol concentration initially while underestimating it during the latter stages of gradient development. Most likely these discrepancies are due to dynamics occurring in the gradient system which are not accommodated in the simulation model. These include volume fluctuations in mixing chambers 1 and 3 during initial stages of the gradient as well as non-ideal behavior (non-uniform flow between mixing chambers) during the order-reduction transition phases.

Butanol concentration was determined with a gas-liquid chromatograph[19] (GLC) equipped with a hydrogen-flame detector. A 183 cm x 2 mm I.D. glass, U-shaped column was filled with 10% Hallcominm-18-OL on 80/100 mesh Supelcoport[20]. Operational parameters of the chromatograph and peripherial equipment are listed in Table XVII. The integrator output of the Mark I electrometer was connected to a digital integrator[21] which automatically measured and recorded the area under each chromatographic peak. A strip chart recorder[22] provided visual tracings of the chromatographic events.

The GLC system response was linear over the range of 0.5 to 1.7 µg butanol per injection. Samples of selected column fractions from the LLC system were diluted such that the GLC response was within the linear range. Butanol concentration (% v/v) was calculated from the standard curve and the appropriate dilution factor.

Figure 18. Actual vs Simulated Response of the Continuous Gradient Elution System. Values represent percent n-butanol in the indicated column fraction. Observed, Δ——Δ; Simulated, □——□ .

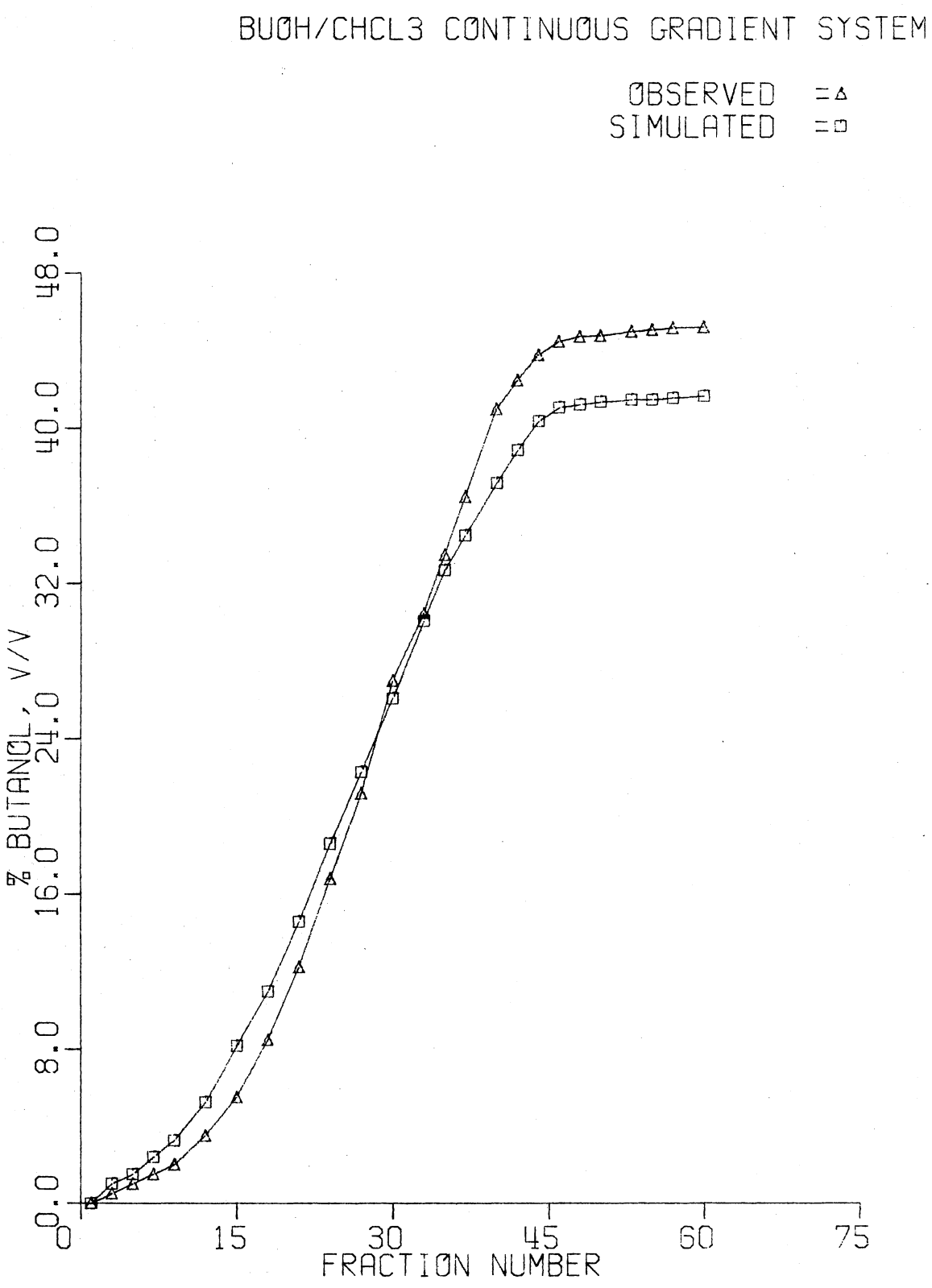

BUØH/CHCL3 CONTINUOUS GRADIENT SYSTEM
ØBSERVED =△
SIMULATED =□
% BUTANØL, V/V
0.0
8.0
16.0
24.0
32.0
40.0
48.0
0
15
30
45
60
75
FRACTION NUMBER

TABLE XVII

OPERATIONAL PARAMETERS OF THE GLC SYSTEM USED IN THE DETERMINATION OF THE CONCENTRATION OF N-BUTANOL IN CHLOROFORM AS GENERATED BY THE CONTINUOUS GRADIENT SYSTEM

Instrument	Bendix 2500
Column length	183 cm
Column I.D.	2 mm
Column type	glass, U-tube
Liquid Phase	10% Hallcominm-18-OL
Packing support	Chromosorb WAW (Supelcoport)
Mesh	80/100
Temperatures	
Column	70°C
Inlet	140°C
Detector	250°C
Gas flow rates	
Nitrogen (lamp grade)	30 ml/min
Hydrogen	27 ml/min
Air	1.6 c.f.h.
Attenuation settings	
Suppression	1K
Input	1
Recorder	1
Integrator settings	
Slope sensitivity	16
Filtering	8
Noise	5
Volume injected	1 µl
Syringe type	Hamilton No. 7002

Isolation of VFAs from Butanol/Chloroform Fractions

Initially the size of the fractions collected from the LLC system was 450 drops. As the gradient developed, fraction volume increased from approximately 3.2 ml to 4.1 ml (ave. 3.8 ml) per column due to the increasing viscosity of the eluting solvent. In order to detect the presence and relative amount of acid(s) present in each fraction, a 1 µl sample from each fraction was injected into a GLC equipped with a 183 cm x 2 mm I.D. glass, U-tube column containing 10% SP-1200/1% H_3PO_4 in Chromosorb W^{20} (Ottenstein and Bartley, 1971). This column, operating according to the GLC operational parameters given in Table XVIII, was used to detect all VFAs except acetic acid. Acetic acid was obscured on the SP-1200 column by the presence of butanol; therefore, a 10% Hallicominm-18-OL column was used to detect the presence of acetic acid in the terminal LLC fractions. This column was operated under the conditions listed in Table XVII except that the column and inlet temperatures were 110 and 160°C, respectively.

Illustrated in Figure 19 is a typical LLC chromatogram of the fractionation of the 48,000 x g supernatant on a silica gel column. The C_5 fraction contained isovaleric, valeric and, sometimes, caproic acid while the C_4 fractions contained isobutyric and butyric acids. Although the tailing of the butyric and propionic acid peaks was slightly less than that obtained with the step gradient system (Figure 16), a marked tailing of acetic acid was still present.

The first and last fractions of each peak were discarded and the remaining fractions of each peak were poured into 55 ml glass test

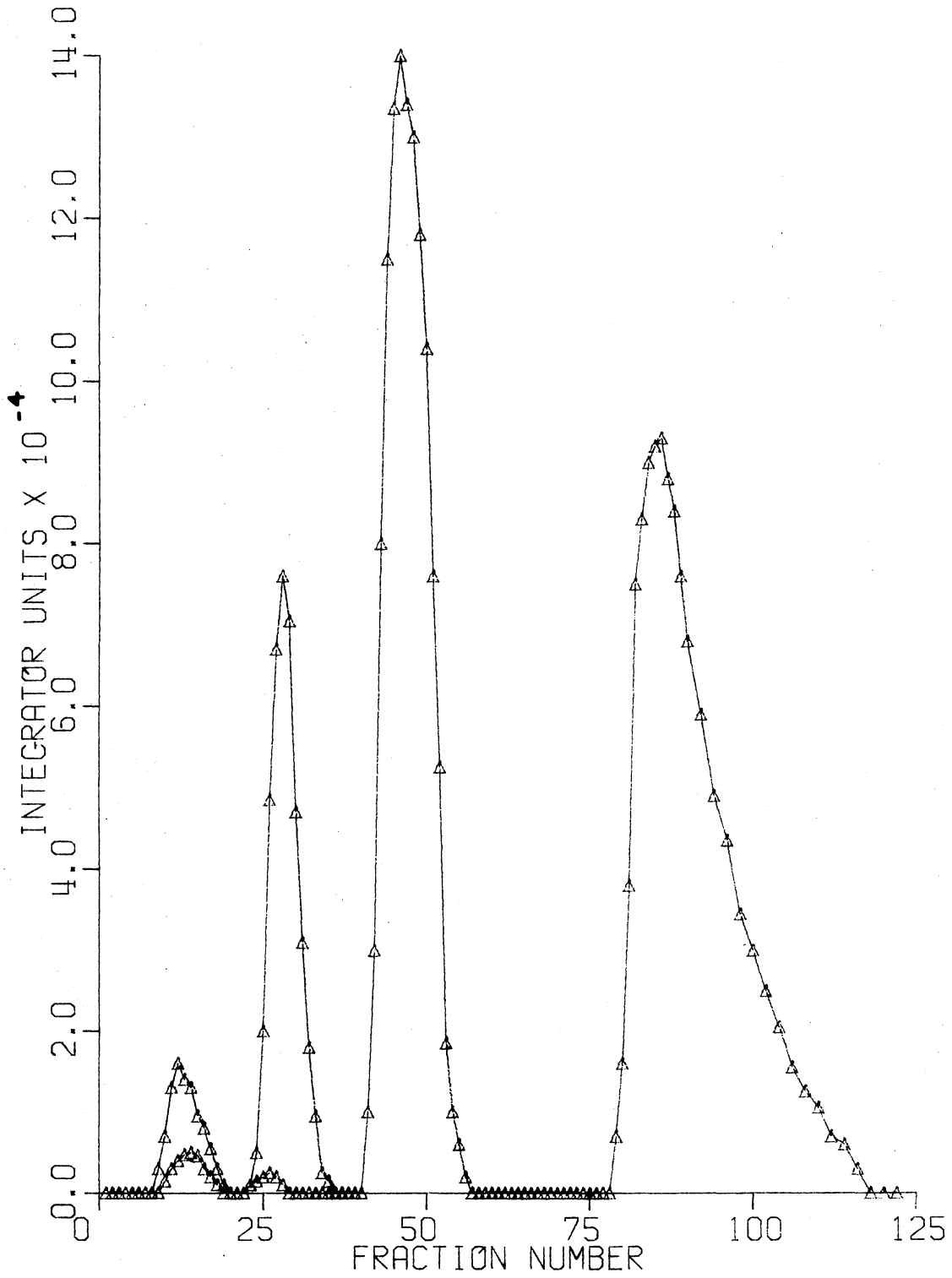
TABLE XVIII

OPERATIONAL PARAMETERS OF THE GLC SYSTEM USED IN THE DETECTION OF VFAS (EXCEPT ACETIC ACID) IN THE BUTANOL/CHLOROFORM LLC FRACTIONS

Instrument	Bendix 2500
Column length	183 cm
Column I.D.	2 mm
Column type	glass, U-tube
Liquid Phase	10% SP-1200/1% H_3PO_4
Packing support	Chromosorb W
Mesh	80/100
Temperature	
Column	
C_5	120°C
C_4	110°C
C_3	100°C
Inlet	180°C
Detector	250°C
Gas flow rates	
Nitrogen (lamp grade)	60 ml/min
Hydrogen	27 ml/min
Air	1.6 c.f.h.
Electrometer settings	Table XVII
Integrator settings	Table XVII
Volume injected	1 µl
Syringe type	Hamilton No. 701

Figure 19. Fractionation of VFAs from Rumen Fluid (04-422-Pr-WC) on a Buffered (pH 7.35) Silicic Acid Column Eluted by a Non-Linear n-Butanol/Chloroform Continuous Gradient System. VFAs were detected by gas chromatography (see text). Fraction size is approximately 4 ml.

BUFFERED SILICIC ACID COLUMN
PH = 7.35 CONTINUOUS GRADIENT SYSTEM



tubes. As a result, four major fractions per column were obtained. These fractions and their contents were: (1) C_5 - isovaleric, valeric and caproic acids, (2) C_4 - isobutyric and butyric acids, (3) C_3 - propionic acid and (4) C_2 - acetic acid. To each fraction (C_2 - C_5) was added 0.3 meq of NaOH[23] in order to form the Na-salt of the carboxylic acids. After mixing, the fractions were allowed to stand for 2 hours. During this time some of the salt formed would settle to the bottom of the tube. This was followed by placing the tubes in a 70°C sand bath and the solvent was evaporated under a stream of dry nitrogen. The temperature of the sand bath was gradually increased to 110°C over a 4 hour period. The fractions were evaporated to complete dryness and then placed in a forced draft oven (105°C) for 2 hours. The fractions were then cooled. This was followed by the addition of 0.5 meq of HCl[24] to convert the carboxylate Na-salt to the carboxylic acid. After mixing the fractions were allowed to stand for about 1 hour before being poured into appropriately labeled glass vials. Fractions were stored at -20°C prior to specific activity determinations.

Specific Activity Measurements

C_2, C_3 and C_4 Fractions. Fractions were thawed and were allowed to reach room temperature before analysis. In duplicate, a 500 µl aliquot of each fraction was pipetted into a 20 ml counting vial, 10 ml of Bray's scintillation solvent was added and the radioactivity counted in a liquid scintillation spectrometer[9]. Radioactivity measurements were corrected for quench by automatic external standardization. Typical counting efficiency was 75%. Radioactivity in each fraction

was expressed as μCi/ml of fraction assayed.

Samples were counted twice, using a 20 minute counting interval, to minimize counting errors. The standard deviation (Faires and Parks, 1973) of the total count of a single sample (or background) was calculated as the square root of the mean count ($\bar{x}$). A 95% confidence interval ($\bar{x} \pm w$) was calculated where

$$w = 1.96 \sqrt{\bar{x}} \quad . \tag{4.3}$$

The relative error, $(w)(100)/\bar{x}$, in the background count was normally 7.5%, but the error in the total sample count ranged from < 1.0% to 7.2%. This may, however, be misleading because VFAs with very low specific activities would exhibit net counts less than or equal to the background count. Because of these discrepancies, the error in the net sample count was calculated as

$$w_{s-b} = \sqrt{w_s^2 + w_b^2} \tag{4.4}$$

This error was expressed relative to the net sample count ($\bar{x}_{s-b}$) such that

$$RE = \frac{w_{s-b}}{\bar{x}_{s-b}} (100) \tag{4.5}$$

where RE is the % random error (95% confidence) in the net sample count.

The quantity of acid present in each fraction, expressed as gram-atoms carbon (gac) per ml, was determined by gas-liquid chromatography. Operating conditions were similar to those listed in

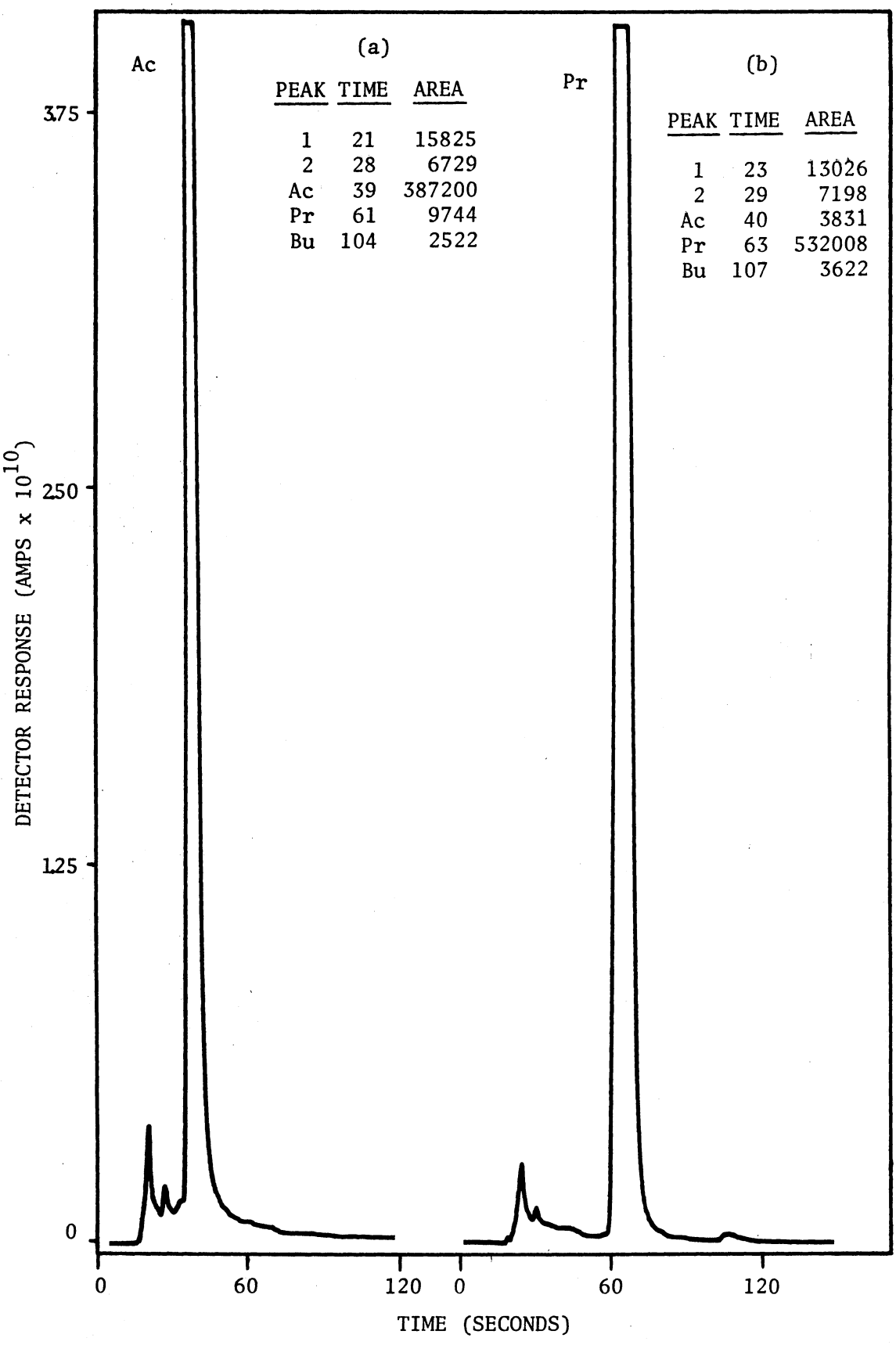
Table XVIII except: column temperature, 115°C; syringe type, No. 7002 Hamilton. Four standards for each acid were used to construct calibration curves during each period of analysis. In all cases, the GLC system response was linear over the concentration range of samples and standards analyzed. Under these conditions, the minimum detectable quantity (MDQ) of acetic, propionic and butyric acid was 300, 150 and 150 pg, respectively. This value was calculated by the equation

$$\text{MDQ } (\mu g) = \frac{2[\text{noise level (amps)}]}{\text{Detector Response (amps}/\mu g)} \quad . \tag{4.6}$$

It should be pointed out, however, that because of detector signal attenuation by the combined effects of the integrator noise, slope sensitivity and filtering controls, the actual minimum detectable quantity (AMDQ) was approximately 10 times MDQ.

Generally, each fraction, except for C_4, possessed only one detectable VFA peak. However, occasionally detectable quantities of the other VFAs would also be present (Figure 20 a,b,c). For example, the C_2 fraction (Figure 20 a) would contain largely acetic acid, but, at times, detectable amounts of propionic and/or butyric acids would be found. Likewise, the C_3 fraction (Figure 20 b) would consist primarily of propionic acid, but would sometimes contain detectable quantities of acetic and/or butyric acids. The C_4 fraction although primarily butyric acid, usually contained a significant quantity of isobutyric acid; however, its concentration was generally $< 1.0\%$ of the butyric acid peak (Figure 20 c). In addition, there was occasionally detectable amounts of propionic, isovaleric and/or valeric acids. In all calculations, the presence of isobutyric

Figure 20. Gas-Liquid Chromatography of the C_2, C_3 and C_4 Fractions from Sample 07-608-Ac-WC. (a) C_2, (b) C_3, (c) C_4. Column: SP-1200, Column temperature: 115°C, N_2 flow: 60 ml/min, Attenuation: 5×10^{-11} amps/inch, Background noise level: 7×10^{-14} amps, Volume injected: 1 µl. For additional operation data, see Table XVIII.


Ac
Pr
(a)
PEAK TIME AREA
1 21 15825
2 28 6729
Ac 39 387200
Pr 61 9744
Bu 104 2522
(b)
PEAK TIME AREA
1 23 13026
2 29 7198
Ac 40 3831
Pr 63 532008
Bu 107 3622
375
250
125
0
DETECTOR RESPONSE (AMPS x 10^{10})
0
60
120
0
60
120
TIME (SECONDS)

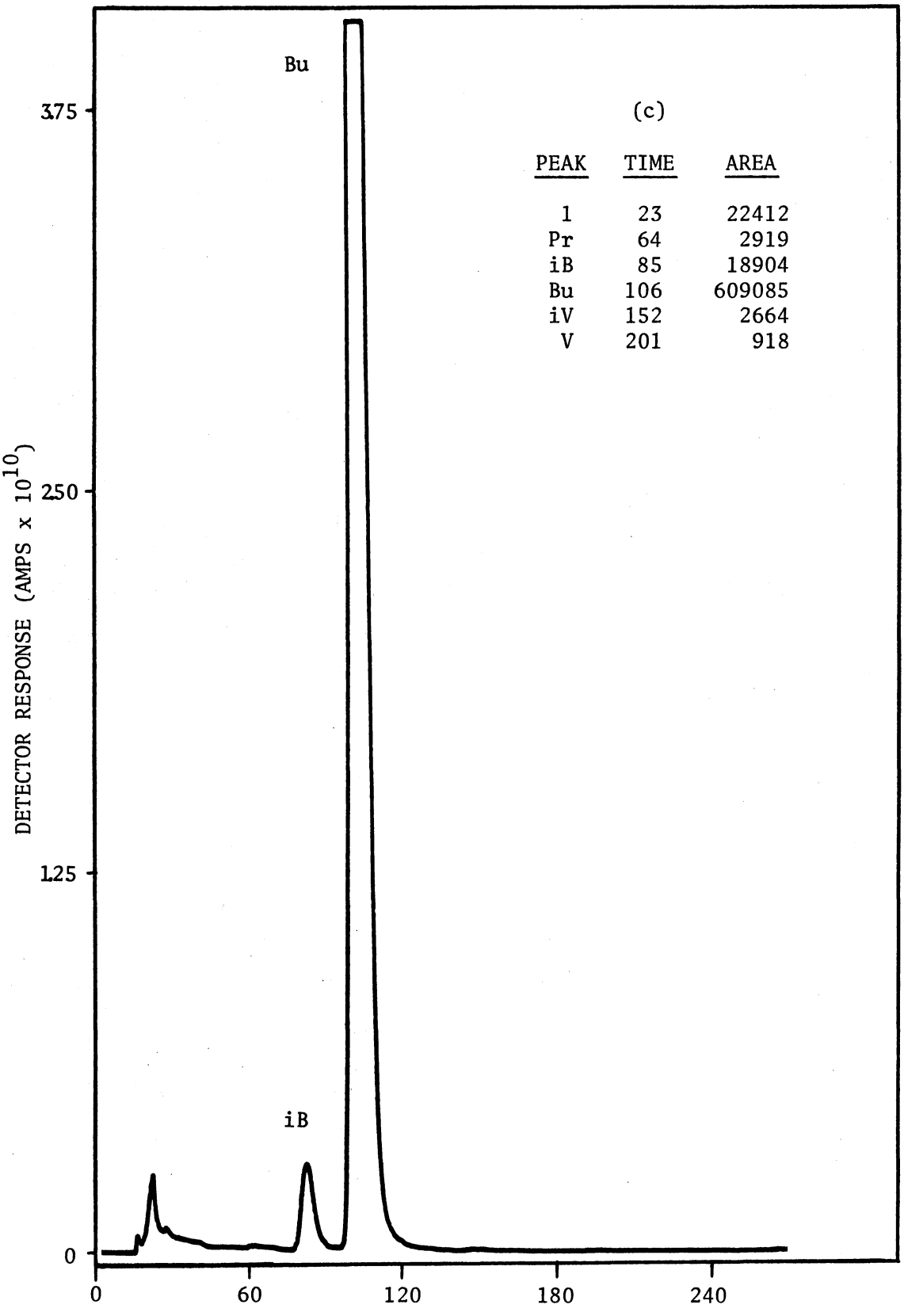


Figure 20. (Continued)

isovaleric and valeric acids in the C_4 fraction was ignored and, furthermore, it was assumed that a negligible quantity of the radioactivity in C_4 came from these acids.

When detectable quantities of extraneous VFA(s) were present, the concentration of radioactivity in that fraction was corrected for the amount of radioactivity contributed by the contaminating VFA(s). Usually this correction was insignificant (< 1-3% change); however, when the specific activity of the extraneous VFA(s) was high when compared to the primary VFA (10-50 times), a radioactivity correction of 15-30% could result from a 1% (w/w) contamination.

The specific activity of each acid was calculated as

$$a = \frac{\mu Ci/ml}{gac/ml} = \mu Ci/gac. \qquad (4.7)$$

C_5 Fraction. With this fraction it was necessary to separate the isovaleric and valeric acids (Figure 21) in order to make the appropriate specific activity measurements. This was accomplished by using an SP-1200 column in a preparative gas chromatograph (PGC)[25]. Operational parameters are given in Table XIX. A stream splitter (50:1) was used to collect samples of column effluent into scintillation vials containing 0.5 ml, 0.1 N NaOH (aq) as a trapping agent. The trapping solutions were maintained at approximately 4°C throughout the collection period.

Once a sufficient quantity of acid has been collected (usually 8-10 injections), 0.02 ml of 4 N HCl was added to each collection vial. The concentration of acid in each collection vial was quantitated with the analytical GLC (Bendix 2500). Assay conditions were the same as employed for the C_2 - C_4 fractions except: column

Figure 21. Gas-Liquid Chromatography of the C_5 Fraction from Sample 07-608-Ac-WC. Column: SP-1200, Column temperature: 115°C, N_2 flow: 60 ml/min, Attenuation: 5×10^{-11} amps/inch, Background noise level: 7×10^{-14} amps, Volume injected: 0.6 µl. For additional operational data, see Table XVIII. Note: Volume of the fraction was reduced approximately 10 fold prior to analysis.

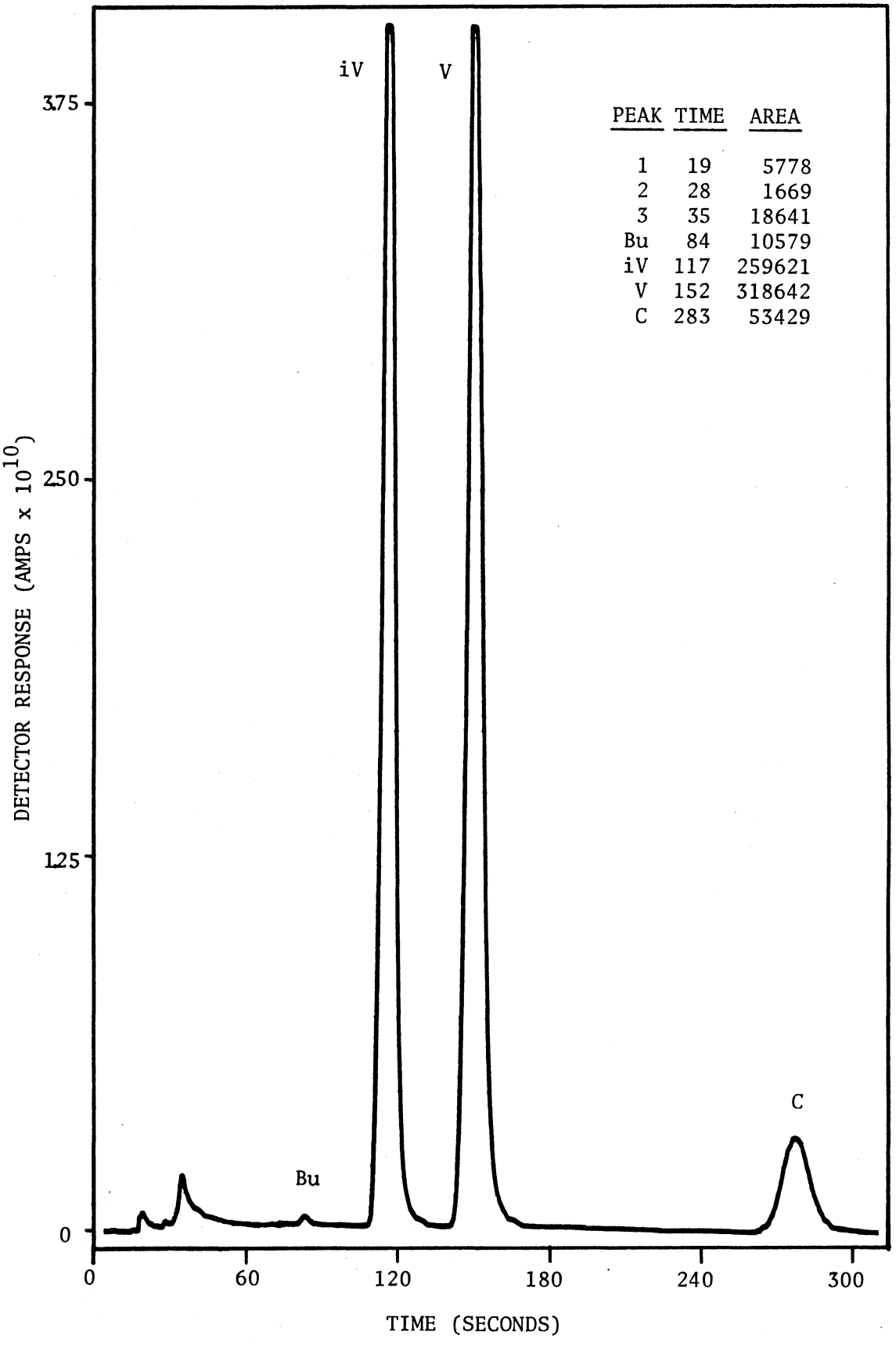

iV
V
Bu
C
PEAK TIME AREA
1 19 5778
2 28 1669
3 35 18641
Bu 84 10579
iV 117 259621
V 152 318642
C 283 53429
3.75
2.50
1.25
0
DETECTOR RESPONSE (AMPS x 10^10)
0
60
120
180
240
300
TIME (SECONDS)

TABLE XIX

OPERATIONAL PARAMETERS OF THE PREPARATIVE GAS CHROMATOGRAPH USED IN THE SEPARATION AND COLLECTION OF ISOVALERIC AND VALERIC ACIDS FROM THE C_5 FRACTION

Instrument	Perkin-Elmer 990
Column length	195 cm
Column I.D.	4 mm
Column type	glass, helical
Liquid phase	10% SP-1200/1% H_3PO_4
Packing support	Chromosorb W
Mesh	80/100
Split ratio	50:1
Temperatures	
Column	115°C
Inlet	200°C
Detector	250°C
Gas flow rates	
Nitrogen	65 ml/min
Hydrogen	30 ml/min
Air	2.2 c.f.h.
Volume per injection	40 µl

temperature, 120°C. Typical chromatograms of the isovaleric and valeric PGC fractions are presented in Figure 22 (a,b). Also, radioactivity determinations were performed similar to those used for fractions C_2 - C_4. Specific activity of each acid (isovaleric and valeric) in the C_5 fraction was calculated according to Equation 4.7.

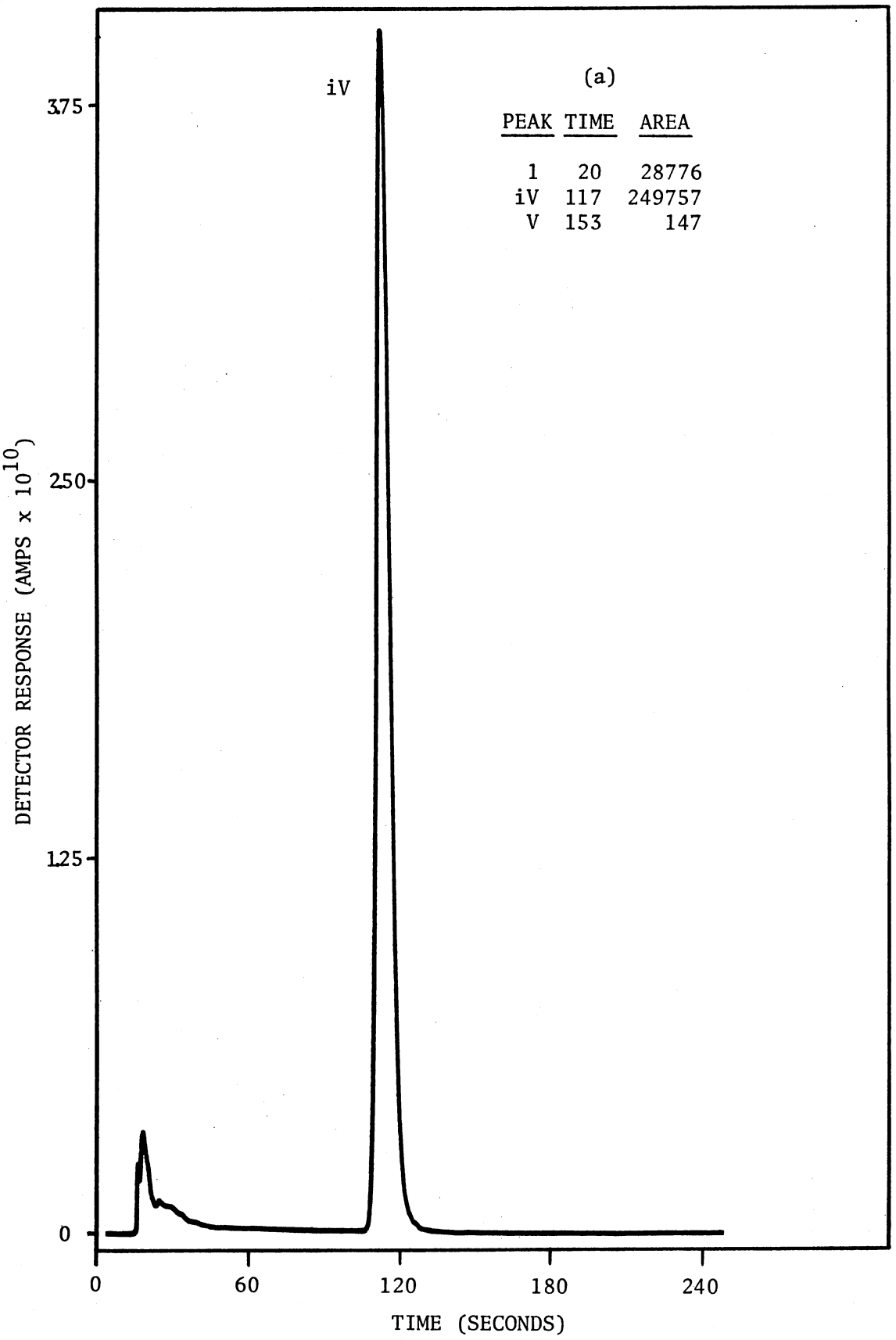
It was suspected that the isovaleric fraction contained both isovaleric isomers; i.e., 2-methylbutyric and 3-methylbutyric acids. Since the SP-1200 column could not distinguish between these isomers, a 3% Carbowax 20 M/0.5% H_3PO_4 column was prepared. Operating conditions for this column are given in Table XX. Standard curves were prepared and the concentration of each isomer was calculated as previously described. However, the specific activity of each isomer was not determined. Typical chromatograms of the isovaleric and valeric PGC fractions, as assayed on the Carbowax column, are illustrated in Figure 23 (a,b).

Rumen Fluid VFA Concentration

The concentration of VFAs in the 48,000 x g supernatant was determined by GLC. The supernatant was adjusted to approximately pH 2 by the addition of 2 drops (about 40 µl) of 12 N HCl to 20 ml of fluid. An SP-1200 column was used in a Bendix 2500 gas chromatograph operating under the conditions listed in Table XVIII with the exception that the column temperature was 120°C.

Two reference standards were prepared, one (WC) having a high Ac:Pr molar ratio (3.70) and the other (GC) having a low Ac:Pr molar ratio (0.923). Samples containing a high Ac:Pr were calculated using the WC standards while those samples having a low Ac:Pr were calcu-

Figure 22. Gas-Liquid Chromatography of the Isovaleric and Valeric PGC Fractions from Sample 07-608-Ac-WC. (a) Isovaleric PGC fraction, (b) Valeric PGC fraction. Column: SP-1200, Column temperature: 120°C, N_2 flow: 60 ml/min, Attenuation: 5×10^{-11} amps/inch, Background noise level: 7×10^{-14} amps, Volume injected: 1 µl. For additional operational data, see Table XVIII.


(a)
PEAK TIME AREA
1 20 28776
iV 117 249757
V 153 147
iV
3.75
2.50
1.25
0
DETECTOR RESPONSE (AMPS x 10^{10})
0
60
120
180
240
TIME (SECONDS)

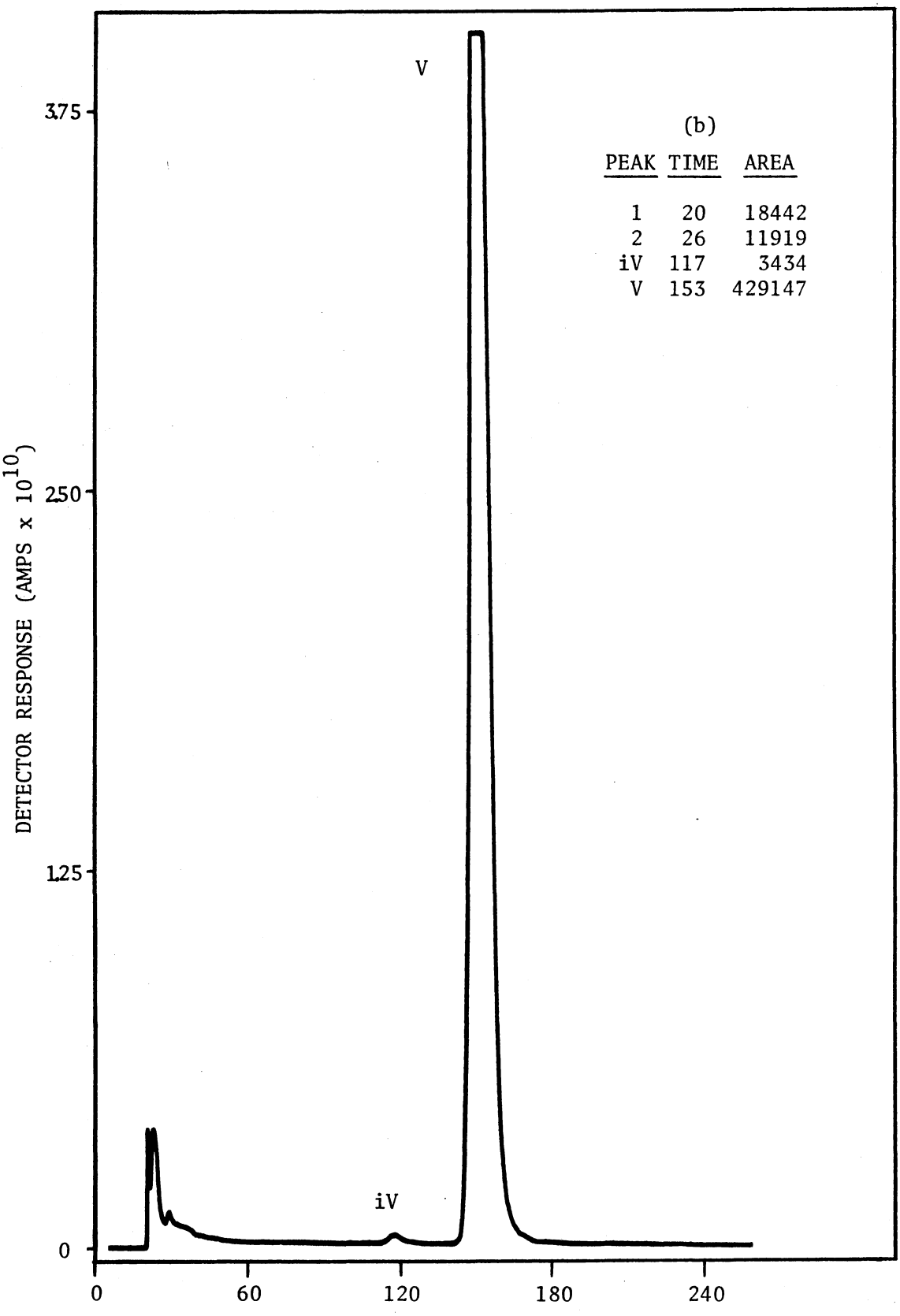


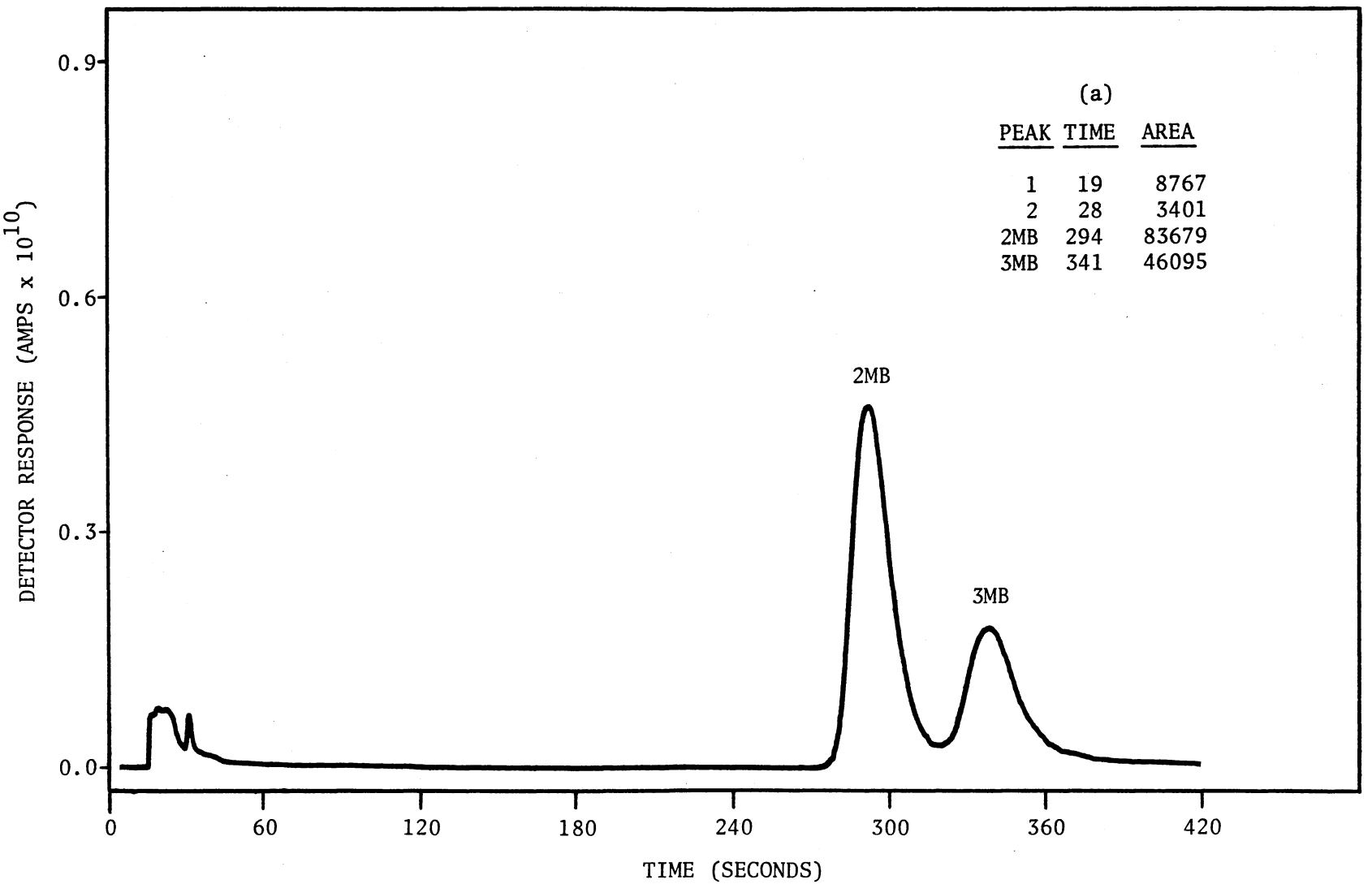
Figure 22. (Continued)

TABLE XX

OPERATIONAL PARAMETERS OF THE GLC SYSTEM USED IN THE DETERMINATION OF 2-METHYL- AND 3-METHYL-BUTYRIC ACIDS

Instrument	Bendix 2500
Column length	183 cm
Column I.D.	2 mm
Column type	glass, U-tube
Liquid phase	3% Carbowax 20 M/0.5% H_3PO_4
Packing support	Carbopack B
Mesh	60/80
Temperatures	
Column	180°C
Inlet	220°C
Detector	285°C
Gas flow rates	
Nitrogen (lamp grade)	60 ml/min
Hydrogen	27 ml/min
Air	1.6 c.f.h.
Electrometer settings	Table XVII
Integrator settings	Table XVII
Volume injected	1 µl
Syringe type	Hamilton No. 7002
Comments	Leave 4 cm headspace w/o glass wool plug

Figure 23. Gas-Liquid Chromatography of the Isovaleric and Valeric PGC Fractions from Sample 07-608-Ac-WC. (a) Isovaleric PGC fraction, (b) Valeric PGC fraction. Column: 3% Carbowax 20 M/0.5% H_3PO_4, Column temperature: 185°C, N_2 flow: 60 ml/min, Attenuation: 2×10^{-11} amps/inch, Volume injected: 1 µl. For additional operational data, see Table XX.


(a)
PEAK TIME AREA
1 19 8767
2 28 3401
2MB 294 83679
3MB 341 46095
2MB
3MB
0.9
0.6
0.3
0.0
0
60
120
180
240
300
360
420
DETECTOR RESPONSE (AMPS x 10^{10})
TIME (SECONDS)

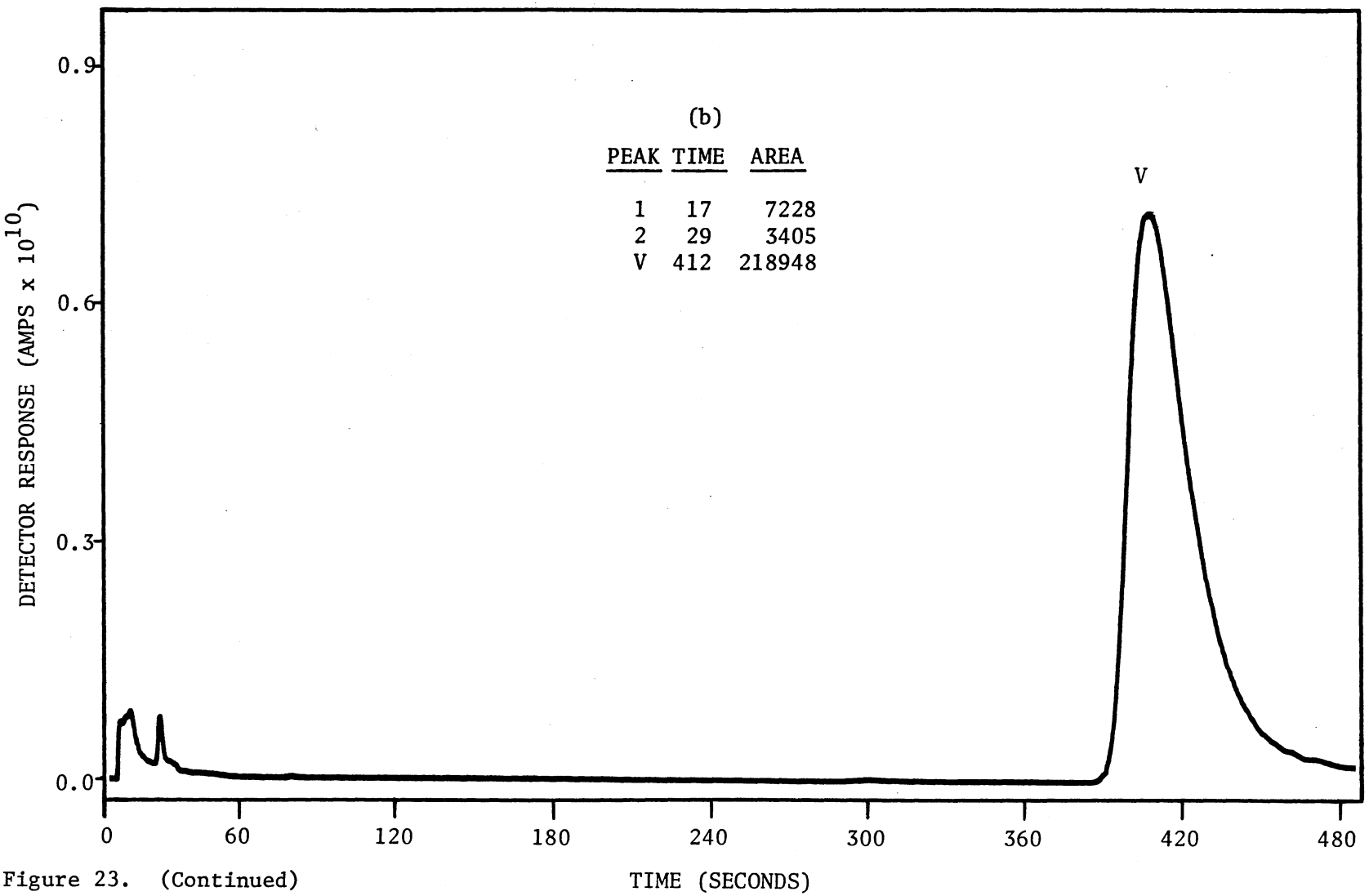


Figure 23. (Continued)

lated with the GC standards. The GLC system was calibrated at three levels of each standard and was found to respond linearly over the concentration range of standards and samples. The GLC-integrator output as a function of VFA concentration for acetic, propionic and butyric acids is shown in Figure 24. Typically, the coefficient of variation (Snedecor and Cochran, 1967) was 3-5%. The VFA concentrations in the standards are given in Table XXI and typical chromatograms of these standards are given in Figure 25 (a,b).

The ratio of 2-methylbutyric (2MB) acid to 3-methylbutyric (3MB) acid in the 48,000 x g supernatant was determined by GLC analysis using the 3% Carbowax Column (Table XX). Only the 4 and 6 hour samples from each infusion were analyzed. The concentration of the 'isovaleric' acid, as determined on the SP-1200 column, was proportioned according to the ratio 2MB:3MB, as determined in the 4 and 6 hour samples. This was assumed to be a valid procedure since 2-methylbutyric and 3-methylbutyric acids gave nearly identical GLC responses on the SP-1200 column.

PEG Analysis

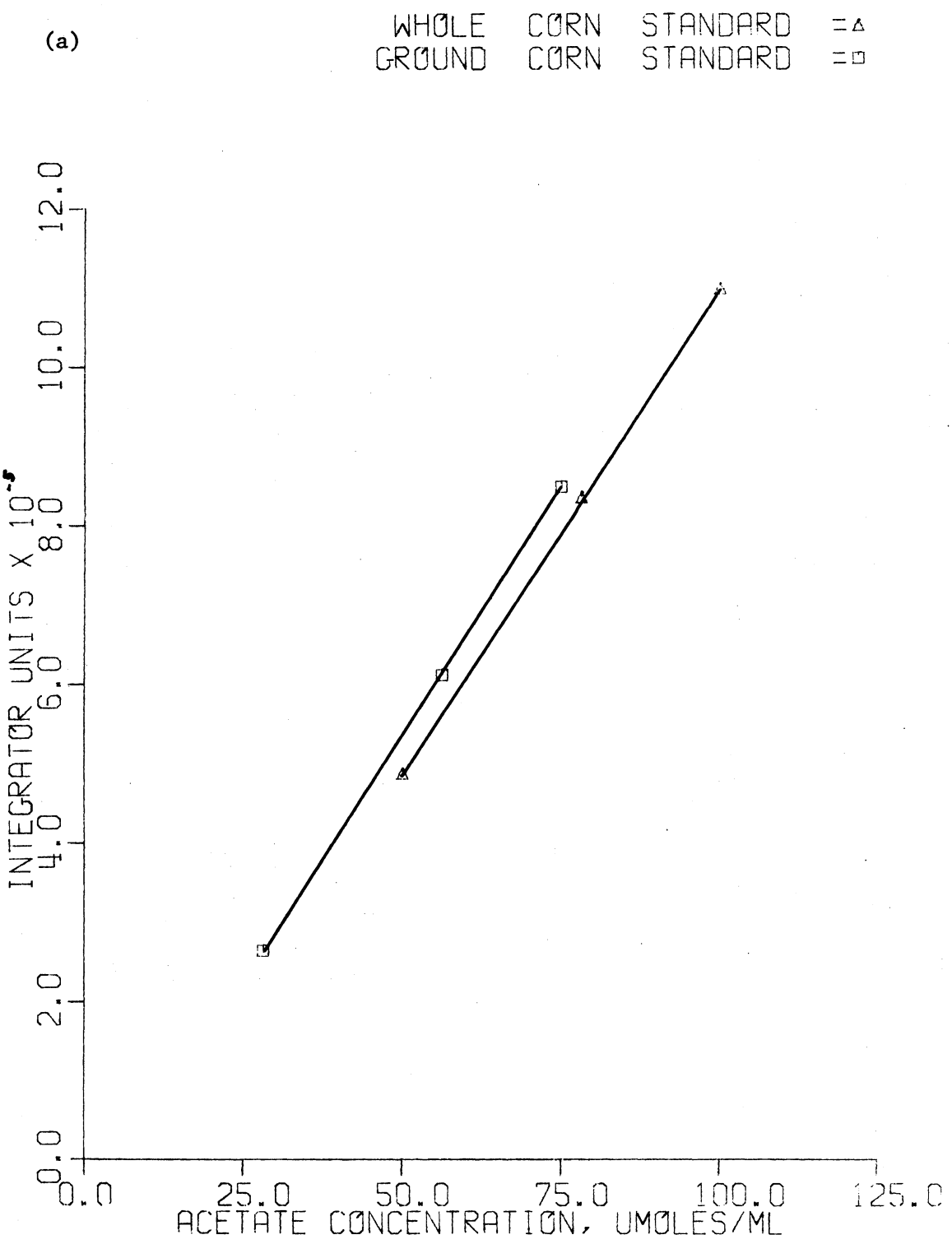
Rumen fluid not treated with mercuric chloride was used for PEG determinations. Rumen samples were thawed and then centrifuged at 12,000 x g for 15 minutes. The 12,000 x g supernatant was used in the PEG assay (Knight, 1971). The experimental procedures followed are described in Table LIII, Appendix C. The turbidometric measurements were conducted with a Gilford Model 240 single beam spectrophotometer[26].

PEG concentration at zero time was calculated according to

Figure 24. GLC-Integrator Response vs VFA Concentration. (a) Acetic acid, (b) Propionic acid and (c) Butyric acid. WC standard; GC standard. For GLC operational data, see Table XVIII and Figure 25.

GLC INTEGRATOR RESPONSE VS ACETATE CONCENTRATION

(a)



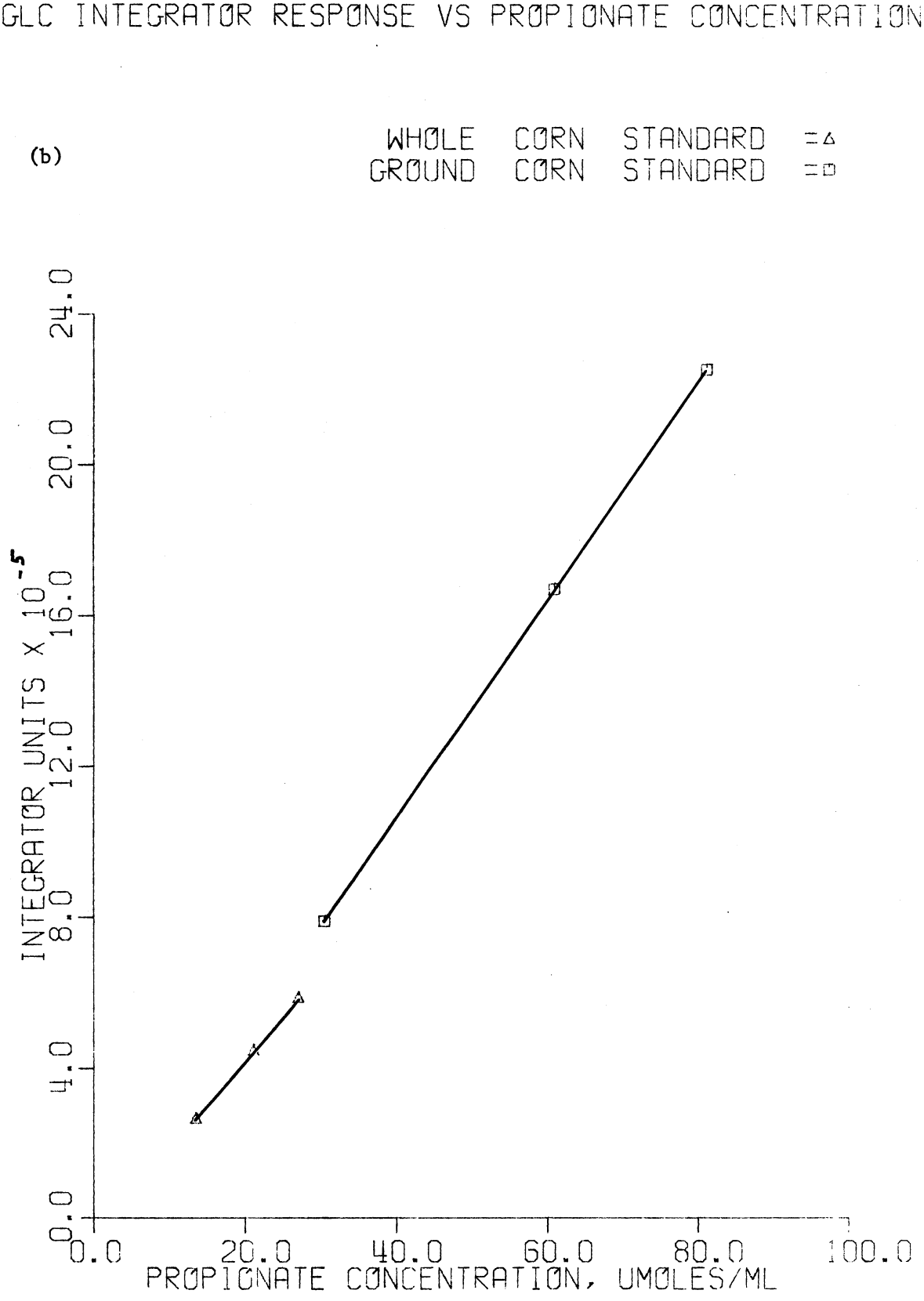


Figure 24. (Continued)

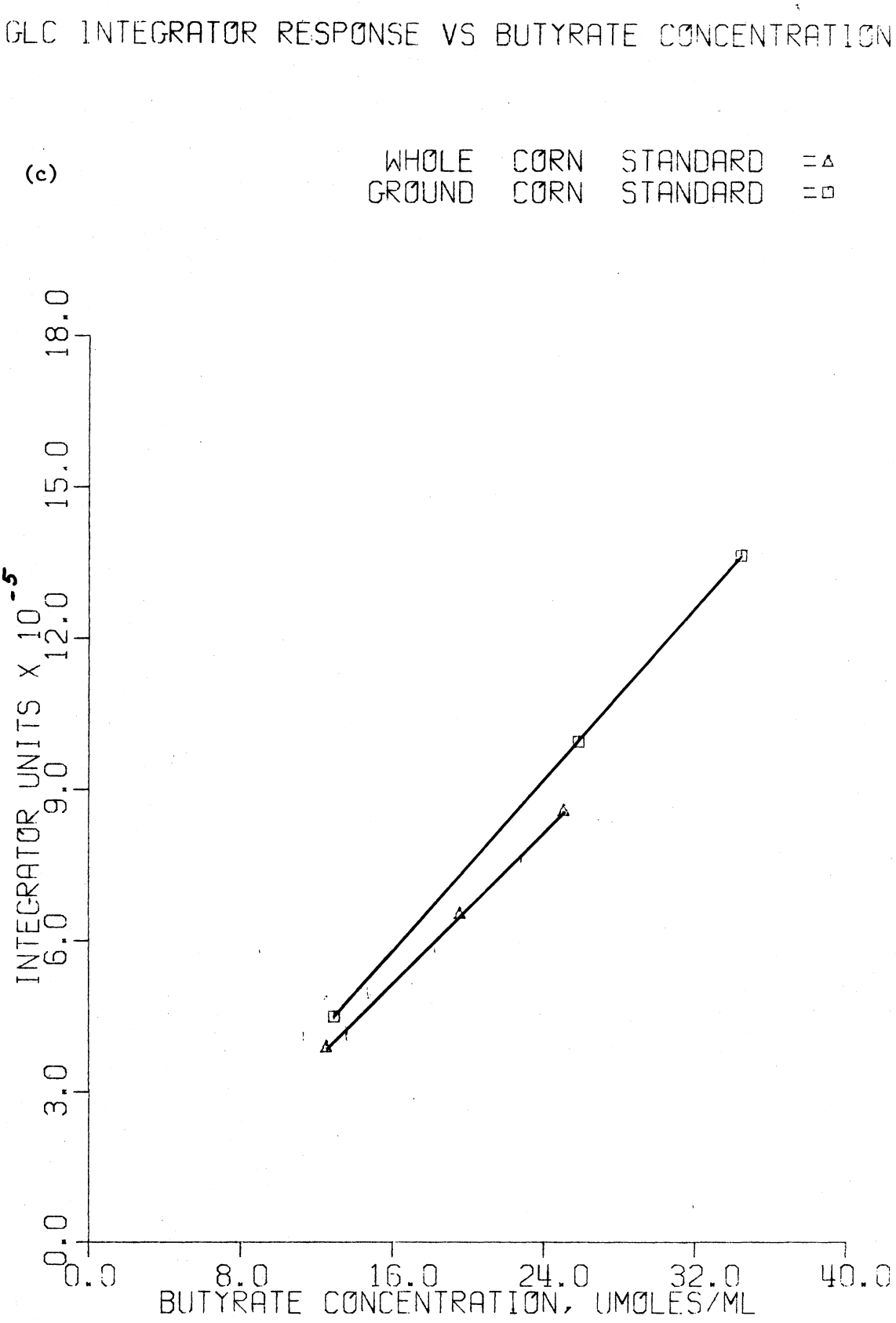


Figure 24. (Continued)

TABLE XXI

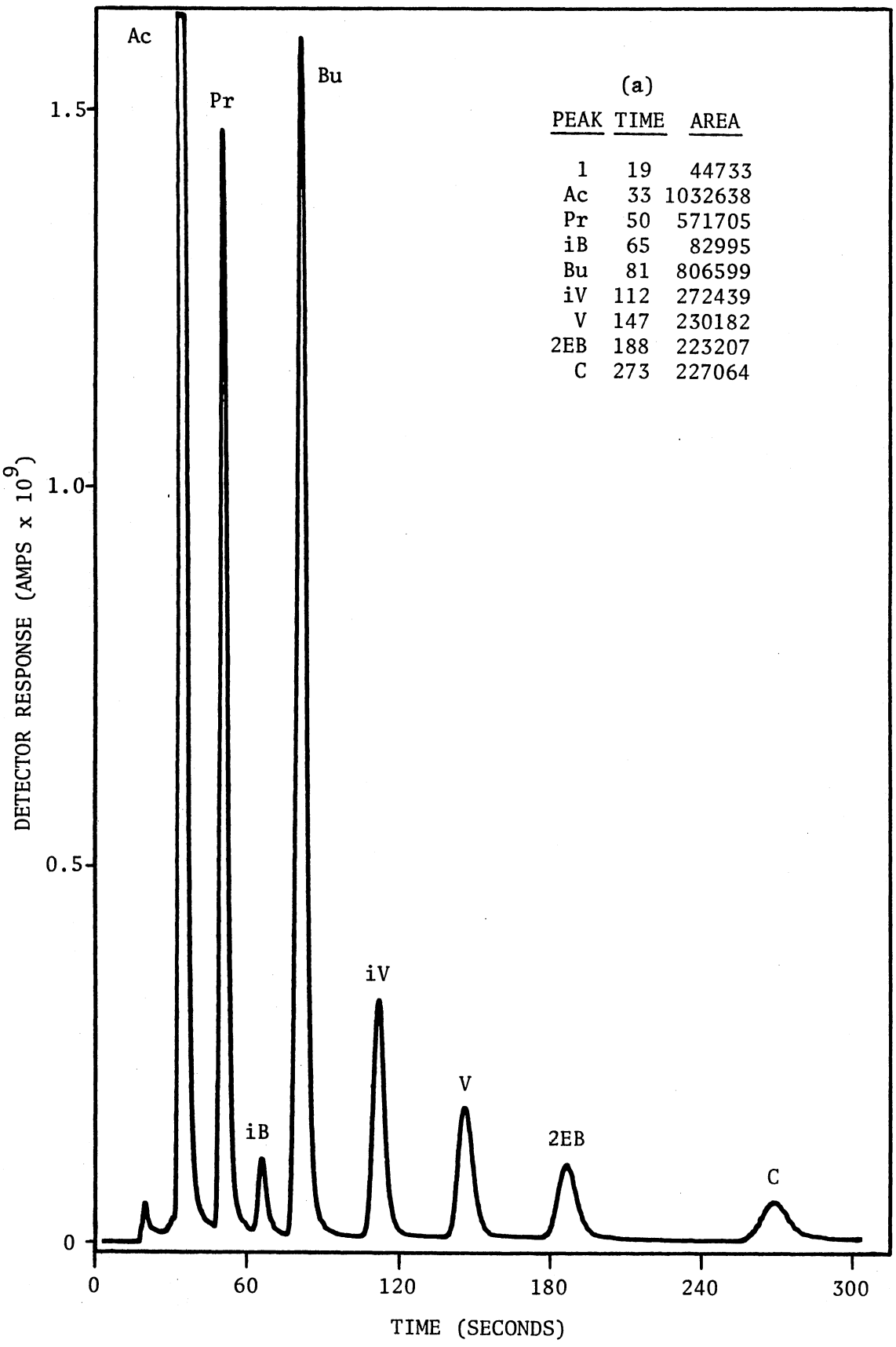
CONCENTRATIONS OF VFAS IN THE WC AND GC STANDARDS[a,b]

Standard	Ac	Pr	iB	Bu	iV	V	2EB	C
WC-1	100.10	27.04	2.275	25.04	6.514	5.220	3.530	2.882
WC-2	78.20	21.13	1.777	19.56	5.090	4.078	2.647	2.252
WC-3	50.05	13.52	1.137	12.52	3.258	2.609	1.765	1.441
GC-1	75.02	81.27	1.877	34.47	3.719	3.708	3.579	2.529
GC-2	56.27	60.95	1.408	25.85	2.790	2.781	2.684	1.897
GC-3	28.13	30.48	0.704	12.93	1.395	1.390	1.342	0.948

[a] µmoles/ml.

[b] Ac: acetic, Pr: propionic, iB: isobutyric, Bu: n-butyric, iV: isovaleric (3-methylbutyric), V: n-valeric, 2EB: 2-ethylbutyric, C: n-caproic.

Figure 25. Gas-Liquid Chromatography of the VFA Standards. (a) Standard WC-2, (b) Standard GC-2. Column: SP-1200, Column temperature: 120°C, N_2 flow: 60 ml/min, Attenuation: 2×10^{-10} amps/inch, Volume injected: 1 µl. For additional operational data, see Table XVIII.


Ac
Pr
Bu
iB
iV
V
2EB
C
(a)
PEAK TIME AREA
1 19 44733
Ac 33 1032638
Pr 50 571705
iB 65 82995
Bu 81 806599
iV 112 272439
V 147 230182
2EB 188 223207
C 273 227064
DETECTOR RESPONSE (AMPS x 10^9)
1.5
1.0
0.5
0
0
60
120
180
240
300
TIME (SECONDS)

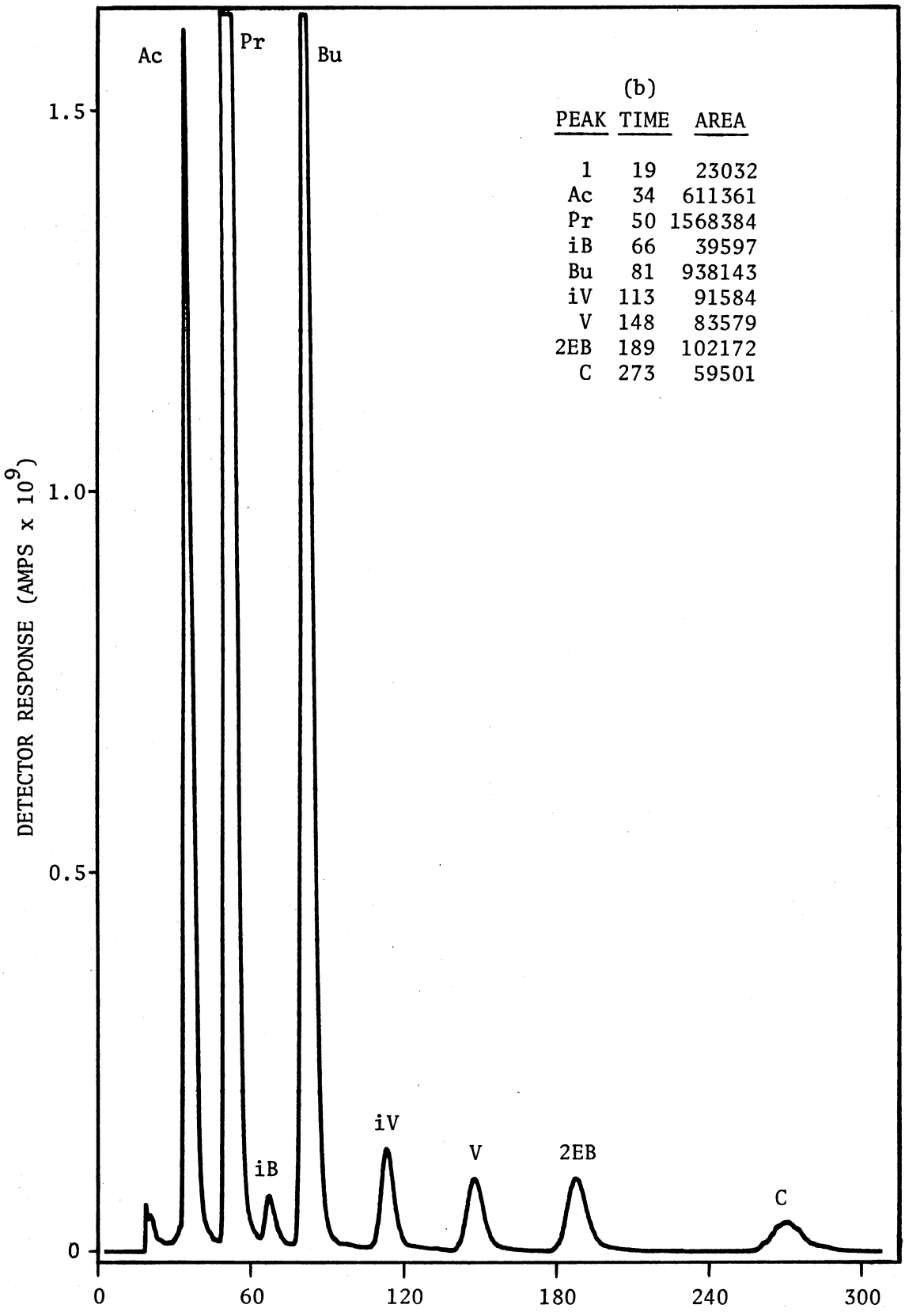


Figure 25. (Continued)

Equation 3.60. Rumen volume and liquid passage to the omasum was determined according to Equations 3.61 and 3.62.

Rumen Fluid Total Radioactivity

Radioactivity per ml of 48,000 x g supernatant was determined by counting in a liquid scintillation spectrometer 1 ml of the 48,000 x g supernatant in 10 ml of 'Kris' scintillation fluid. The formula for the 'Kris' scintillation fluid is given in Table XXII (Kristensen, 1974).

TABLE XXII

FORMULA FOR 'KRIS' SCINTILLATION FLUID[a,b]

Item	Quantity/liter
PPO, (g)[c]	5.0
M_2-POPOP, (g)[c]	0.5
Triton X-100, (ml)[d]	330.0
Toluene[e]	to volume

[a]Kristensen (1974).

[b]All reagents scintillation grade.

[c]Packard Instrument Company.

[d]Amersham/Searle Corporation.

[e]Mallinckrodt Chemical Works.

Quenched standards were prepared using 1 ml of 48,000 x g supernatant, 10 ml of 'Kris', 1-^{14}C-hexadecane (22,500 DMP/vial) and a variable amount (0-200 µl) of chloroform as a quenching agent. Automatic external standardization was used to correct for quench. Nominal counting efficiency was 80%. Values were expressed as µCi per ml of supernatant.

Blood Analysis

Plasma was thawed in a refrigerator and a protein-free supernatant was prepared as outlined in Table LIV, Appendix C. This supernatant was used in the determination of plasma glucose concentration and specific activity.

Plasma Glucose Concentration

Plasma glucose concentration was determined by the glucose oxidase method[27]. Experimental procedures followed are described in Table LIV, Appendix C. The concentration of glucose in the protein-free filtrate was multiplied by the dilution factor of 1.1 to correct for the addition of heparin to whole blood. Plasma glucose concentration was expressed as mg glucose per liter.

Plasma Glucose Specific Activity

Plasma glucose specific activity was determined according to the glucose pentaacetate (GPA) procedure outlined by Jones (1965). The experimental protocol is given in Table LIV, Appendix C. Using 1-^{14}C-D-glucose (53 mCi/mmole)[28], recovery of radioactivity as GPA was 50.2 ± 1.4% (Table XXIII).

TABLE XXIII

RECOVERY OF ^{14}C ACTIVITY WHEN ADDED TO DEPROTEINIZED BOVINE BLOOD

Condition or property	Assay				
	1	2	3	4	5
Activity added as labeled glucose, μCi x 10^{-2}	5.52	5.52	5.52	5.52	5.52
Glucose added via blood filtrate, (616 mg/l), mg	0.616	0.616	0.616	0.616	0.616
Glucose added via label, mg	0.0	0.0	0.0	0.0	0.0
Carrier glucose added, mg	102.2	101.8	102.3	101.3	102.2
Total glucose present, mg	102.8	102.4	102.9	101.9	102.8
Theoretical yield of GPA, mg	222.7	221.8	222.9	220.8	222.7
GPA recovered, mg	114.7	108.9	114.0	110.1	114.9
Chemical recovery, %	51.5	49.1	51.1	49.9	51.6
DPM/vial	61558	59090	61712	61805	63667
Specific activity of GPA, μCi x 10^{-4}/mg	2.42	2.44	2.44	2.53	2.50
Total activity recovered, μCi x 10^{-2}	2.77	2.66	2.78	2.78	2.87
^{14}C activity recovered, %	50.2	48.2	50.4	50.4	52.0
Specific activity of blood glucose found, μCi x 10^{-2}/mg					
By average recovery method	8.84	8.88	8.89	9.00	8.99
By Equation 4.8 (a)	8.75	8.79	8.83	9.07	9.04
Specific activity of blood glucose, theoretical (b), μCi x 10^{-2}/mg	8.96	8.96	8.96	8.96	8.96
$\lvert a - b \rvert / a \times 100$	2.40	1.93	1.47	1.21	0.89

Specific activity of plasma glucose was calculated as

$$a_g = \left[\frac{G_c + G_s}{G_s} \right] \times 2.1663 \times a_{gpa} \qquad (4.8)$$

where a_g is the specific activity of plasma glucose, a_{gpa} is the specific activity of the crystallized GPA, G_c is the amount of 'cold' carrier glucose added and G_s represents the amount of glucose added in 20 ml of protein-free supernatant. The derivation of this equation is given in Appendix D.

Statistical Analysis

Parameter means within a given animal-ration period are accompanied by either their respective standard deviations (s) or coefficients of variation (% CV). Parameter means for a given diet are accompanied by their respective standard errors (SE) calculated as

$$SE = \sqrt{\frac{s^2}{n}} \qquad (4.9)$$

where n is the number of observations in that particular mean.

Differences between animals within diet and between diets were tested using the Student's "t" test (two-tailed) for samples of equal and unequal sizes (Snedecor and Cochran, 1967). The equations used are:

$$SE_d = \sqrt{s^2 \frac{n_1 + n_2}{n_1 n_2}} \qquad (4.10)$$

where SE_d is the standard error of the difference between means $\bar{x}_1$ and $\bar{x}_2$, s^2 is the estimated variance, n_1 is the number of observations in group one and n_2 is the number of observations in group two. The degrees of freedom (df) becomes

$$df = (n_1 - 1) + (n_2 - 1). \tag{4.11}$$

The "t" test was calculated as

$$t = \frac{\bar{x}_1 - \bar{x}_2}{SE_d} . \tag{4.12}$$

FOOTNOTES

[1]Clay Equipment Corporation, Cedar Falls, Iowa 50613.

[2]Eriez Magnetics, Asbury Road at Airport, Erie, Pennsylvania 16512.

[3]Tork Program Signal Timer, Model 5701, Tork Time Controls Inc., Mt. Vernon, New York.

[4]International Chemical and Nuclear Corporation, Irvine, California 92664.

[5]Technical Brochure 64D1, July, 1969, Schwarz/Mann BioResearch, Orangeburg, New York 10962.

[6]Q1, silica gel, 5 x 20 cm, layer thickness - 250 μ ± 4%; Quantum Industries, Fairfield, New Jersey.

[7]Hamilton Company, Reno, Nevada 89510.

[8]Kodak, Medical, No-Screen, Eastman Kodak Company, Rochester, New York.

[9]Packard Tricarb Model 3320, Packard Instrument Company Inc., 2200 Warrenville Road, Downers Grove, Illinois 60515.

[10]Polyethylene glycol, Matheson, Coleman and Bell, Manufacturing Chemists, Norwood, Ohio 45212.

[11]Intramedic polyethylene tubing (PHF), Clay Adams, Parsippany, New Jersey 07054.

[12]Harvard Syringe Pump Model 940, Harvard Apparatus Company Inc., 150 Dover Road, Millis, Massachusetts 02054.

[13]Fischer and Porter Company, Lab-Crest Scientific Division, Warminster, Pennsylvania 18974.

[14]Technicon Industrial Systems, Tarrytown, New York 10591.

[15]Mallinckrodt Chemical Works, St. Louis, Missouri 63160.

[16]Mixture of 2 M NaH_2PO_4 and 2 M K_2HPO_4, Mallinckrodt, reagent grade.

[17]All chloroform used was previously washed three times with glass distilled water, Mallinckrodt (AR), ACS grade.

[18]n-butanol (reagent grade) obtained commerically (Matheson, Coleman and Bell) was distilled in glass to remove contaminating quantities of butyric acid.

[19]Series 2500, Bendix Corporation, Ronceverte, West Virginia 24970.

[20]Supelco, Supelco Park, Bellefonte, Pennsylvania 16823.

[21]AutoLab 6300-02, Vidar, 77 Ortega Avenue, Mountain View, California 94041.

[22]Electronik 194, Honeywell, Industrial Division, Fort Washington, Pennsylvania 19034.

[23]NaOH solution: 1 ml of 0.3 N NaOH in methyl alcohol.

[24]HCL solution: 3 ml of 0.16 N HCl in glass distilled water.

[25]Perkin-Elmer 990 Gas Chromatograph, Perkin-Elmer, Norwalk, Connecticut.

[26]Gilford Instrument Laboratories Inc., Oberlin, Ohio 44070.

[27]Glucostat, Worthington Biochemical Corporation, Freehold, New Jersey 07728.

[28]New England Nuclear Corporation, 549 Albany Street, Boston, Massachusetts 02118.

CHAPTER V

RESULTS AND DISCUSSION

Introduction

This chapter will be divided into two main sections. The first part will deal with the presentation and discussion of the experimental observations associated with the various rumen and blood parameters that were measured. In the last section some of these data will be incorporated into compartment models describing the production and interconversion of volatile fatty acids. Finally, the influence of the physical form of the corn on these model parameters will be discussed.

Experimental Data

Feed Consumption

Dry matter intake (DMI) during each experiment and during the three day period just prior to each experiment are given in Table XXIV. Steers fed the whole corn (WC) ration appeared to have higher DMIs (3 day pre-experimental period) than the steers consuming the ground corn (GC) ration. This is in agreement with other ad libitum feeding trials (Vance et al., 1972; Rounds, 1973; White et al., 1975 a,b) comparing high-concentrate diets containing either whole or ground corn. In addition, feed consumption appeared to be less eratic

TABLE XXIV

DRY MATTER INTAKE DURING EACH EXPERIMENT AND DURING THE THREE DAY PERIOD PRIOR TO EACH EXPERIMENT

Diet	Phase	Animal	Expt	DM Intake		
				3 Days Prior		During Expt
				g/d	g/hr	g/hr
Ground Corn	I	608	Ac	6720	280	215
			Pr	6720	280	280
			Bu	6720	280	280
Ground Corn	II	612	Ac	6553	273	215
			Pr	6553	273	273
			Bu	6553	273	273
$\bar{x}$				6636.5	276.5	256.0
$s_{\bar{x}}$				37.3	1.6	13.0
Whole Corn	I	422	Ac	6775	283	283
			Pr	6775	283	283
			Bu	6775	283	283
Whole Corn	II	608	Ac	8023	334	334
			Pr	7130	297	200
			Bu	7130	297	191
$\bar{x}$				7101.3	296.2	262.3
$s_{\bar{x}}$				197.5	8.1	22.7

on the WC diet resulting in fewer 'off feed' incidents. Also, steers on the WC diet appeared to be less sensitive to fluctuations in feed intake. These fluctuations were usually attributed to feeder malfunction, feeder stoppage, improper feeder control settings or power outage. In general, feed consumption was fairly uniform over the experimental period. However, during the period Whole Corn-Phase II, it seemed as though Animal 608 would plateau at 8023 g DMI per day. This level was maintained for the acetate infusion, but could not be sustained for the other two experiments.

Although attempts were made to insure uniform intake within each ration-animal period, the activities associated with tracer administration and sample collection seemed to influence feed consumption during some of the experiments. These effects are indicated by the fluctuations in DMI as shown in Table XXIV. In addition, it was quite often observed that even though feed consumption was normal during the experimental time frame, considerable amounts of feed remained in the trough the next morning. Therefore, hourly intakes, as measured during the experimental period, were used in subsequent calculations and adjustments of data.

Animal Weight

As pointed out earlier, animals were weighed before and after each animal-ration period. Therefore, to obtain the weight of an animal during a given infusion, a linear extrapolation was made between the initial and final period weight. These weights are presented in Table XXV.

TABLE XXV

ANIMAL WEIGHT DURING EACH INFUSION[a]

Phase	Animal	Ratio	Expt	Kg
I-1	608	GC	Ac	351
			Pr	362
			Bu	356
I-2	422	WC	Ac	373
			Pr	386
			Bu	379
II-1	608	WC	Ac	382
			Pr	404
			Bu	395
II-2	612	GC	Ac	461
			Pr	448
			Bu	453

[a]Obtained by linear extrapolation between the initial and final period weights shown in Table IX.

Rumen Parameters

Rumen Liquid Volume and Turnover Rate Constant. The volume of the rumen liquid equilibrating with PEG and the rate of flow of this liquid from the rumen to the omasum was estimated mathematically according to procedures outlined in Chapter III of this manuscript. The natural logarithm of the rumen PEG concentration (mg/ml) versus time during each experiment is shown in Figures 26-29. The data from each infusion experiment was fitted to the equation

$$\ln \text{PEG}(t) = \ln \text{PEG}(0) - k_m t \qquad (5.1)$$

by a least squares linear regression algorithm where the intercept, PEG(0), is the natural logarithm of the theoretical PEG concentration at zero time, assuming instantaneous mixing of the marker, and k_m is the rate constant associated with the outflow of liquid (PEG) from the rumen to the omasum. It is apparent from the initial portion of most of the PEG time curves that mixing was not instantaneous. Therefore, these initial data points, usually the one and/or two hour samples, were not included in the calculations. The zero time PEG concentration was adjusted for any residual PEG determined in the zero hour sample. This adjusted zero time PEG concentration (mg/ml), M_o, was used to calculate the volume of liquid equilibrating with the PEG according to the equation

$$V_k = \frac{D}{M_{ok}} \qquad (5.2)$$

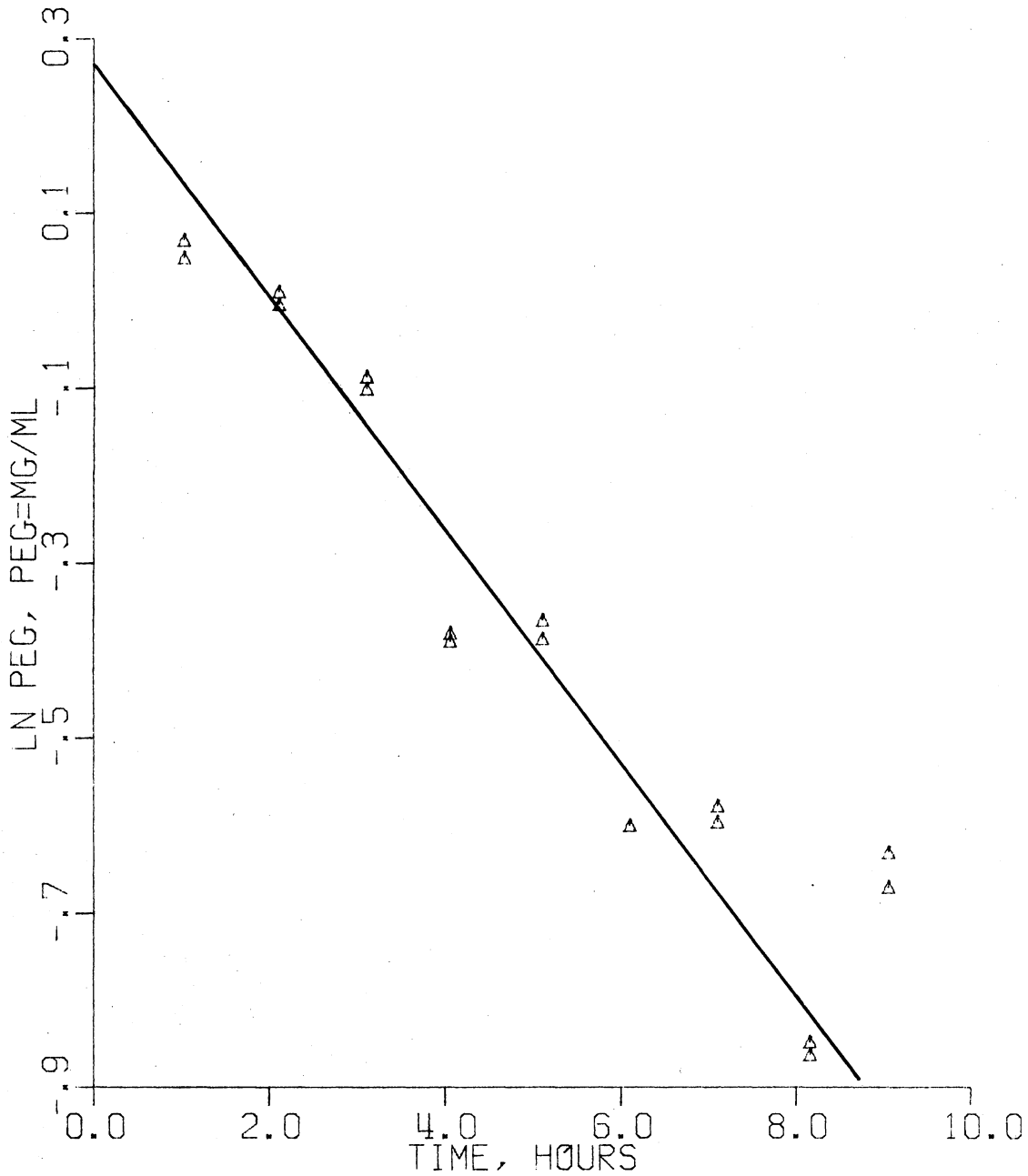
where V_k is the rumen liquid volume (ml) during the k^{th} experiment and

Figure 26. The Natural Logarithm of Rumen PEG Concentration versus Time for Animal 608 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)

LN PEG VS TIME

ANIMAL = 608 RATION = WC EXPT = AC △
LN PEG = 0.2717 - 0.1338 T
PEG(0) = 0.0518



(b)

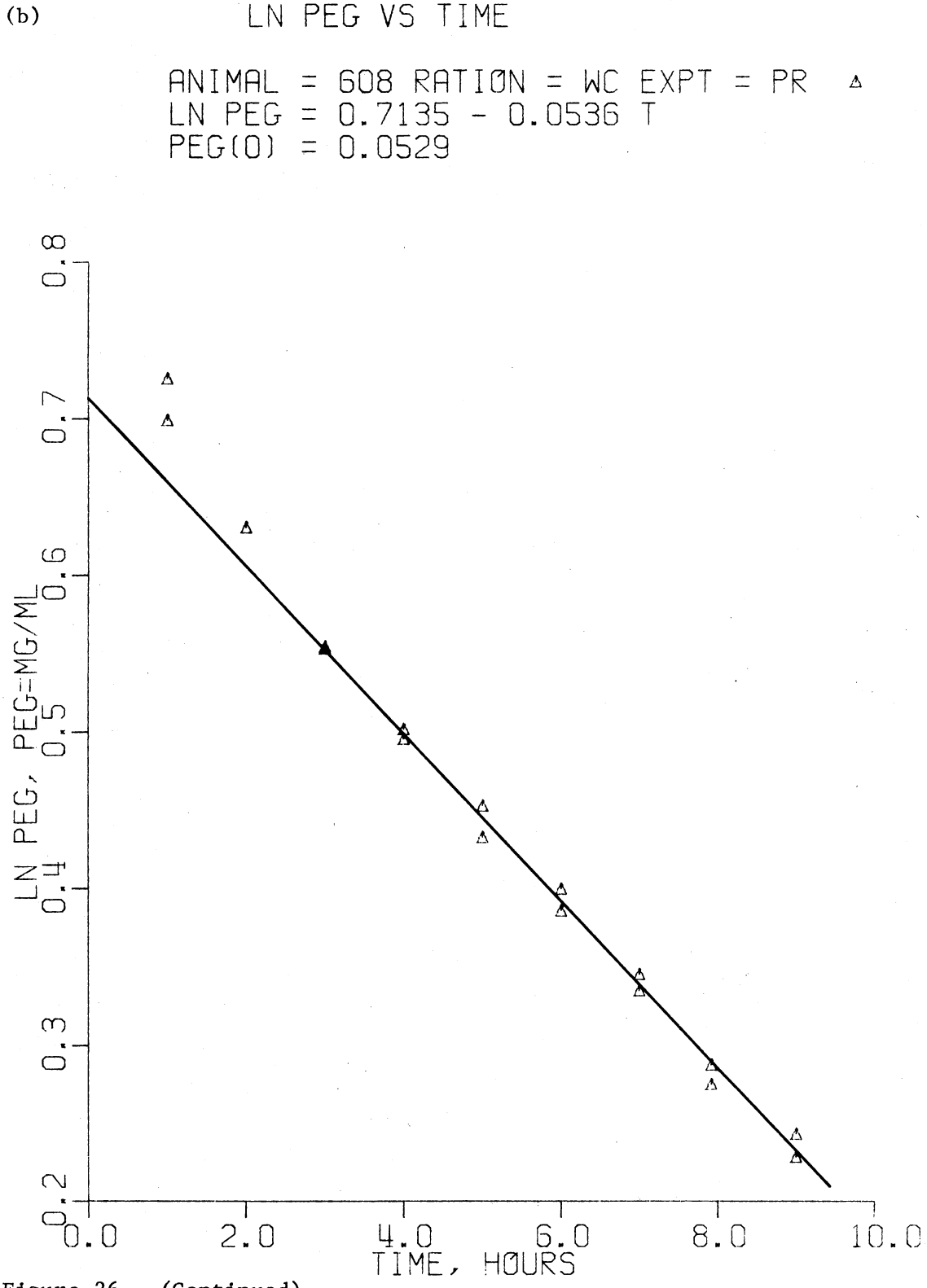


Figure 26. (Continued)

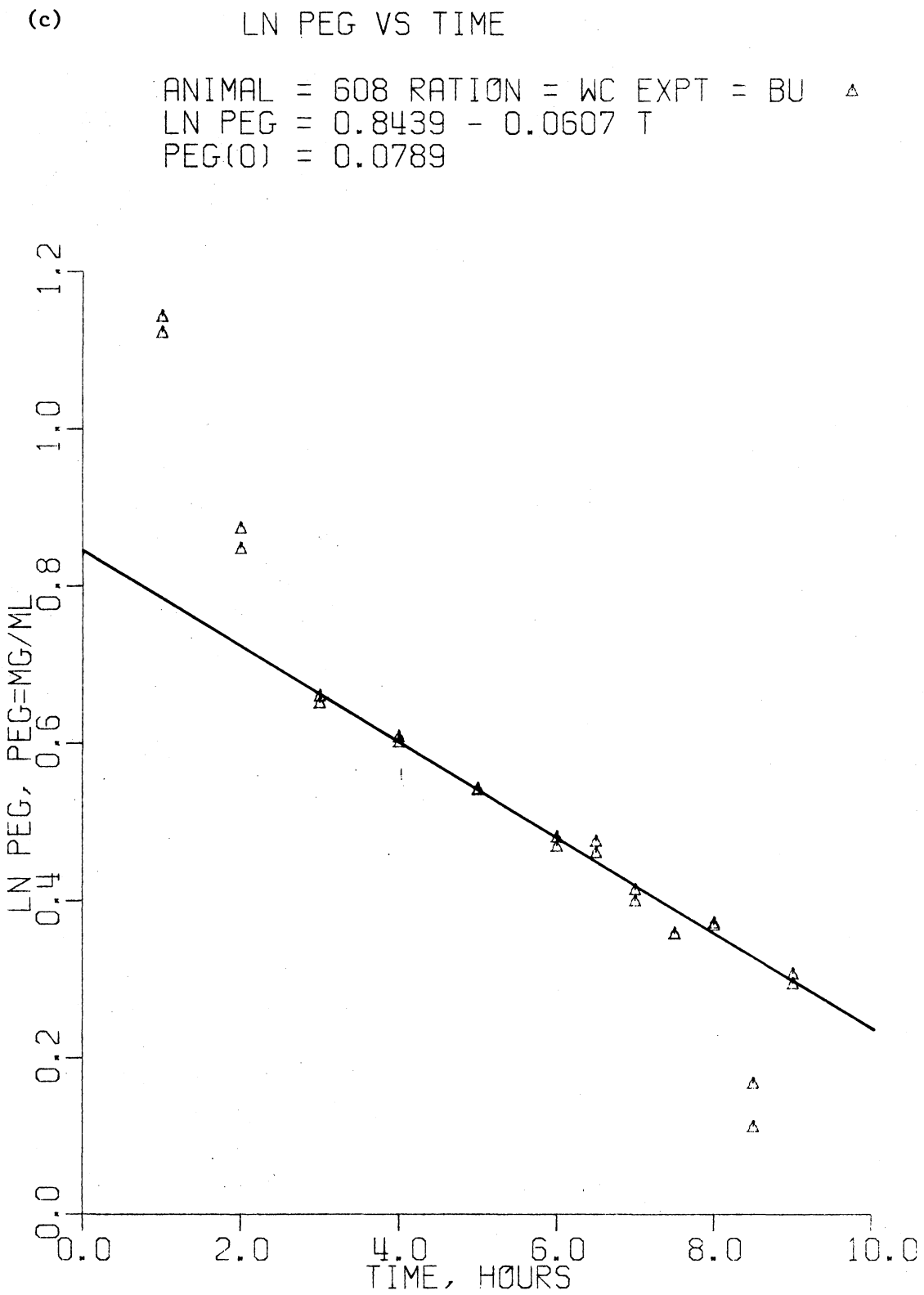


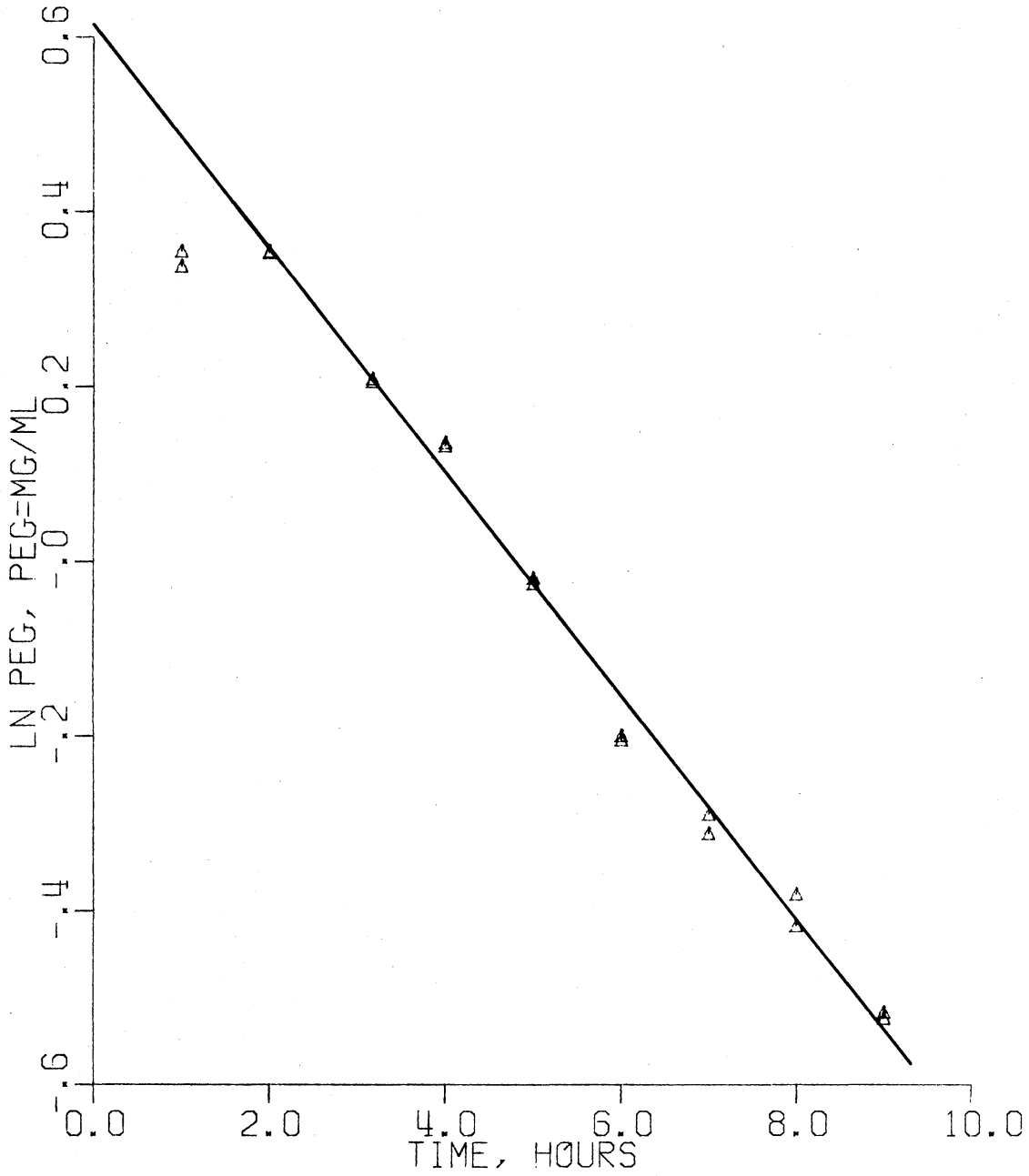
Figure 26. (Continued)

Figure 27. The Natural Logarithm of Rumen PEG Concentration versus Time for Animal 422 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)

LN PEG VS TIME

ANIMAL = 422 RATION = WC EXPT = AC △
LN PEG = 0.6134 - 0.1279 T
PEG(0) = 0.0765



(b)

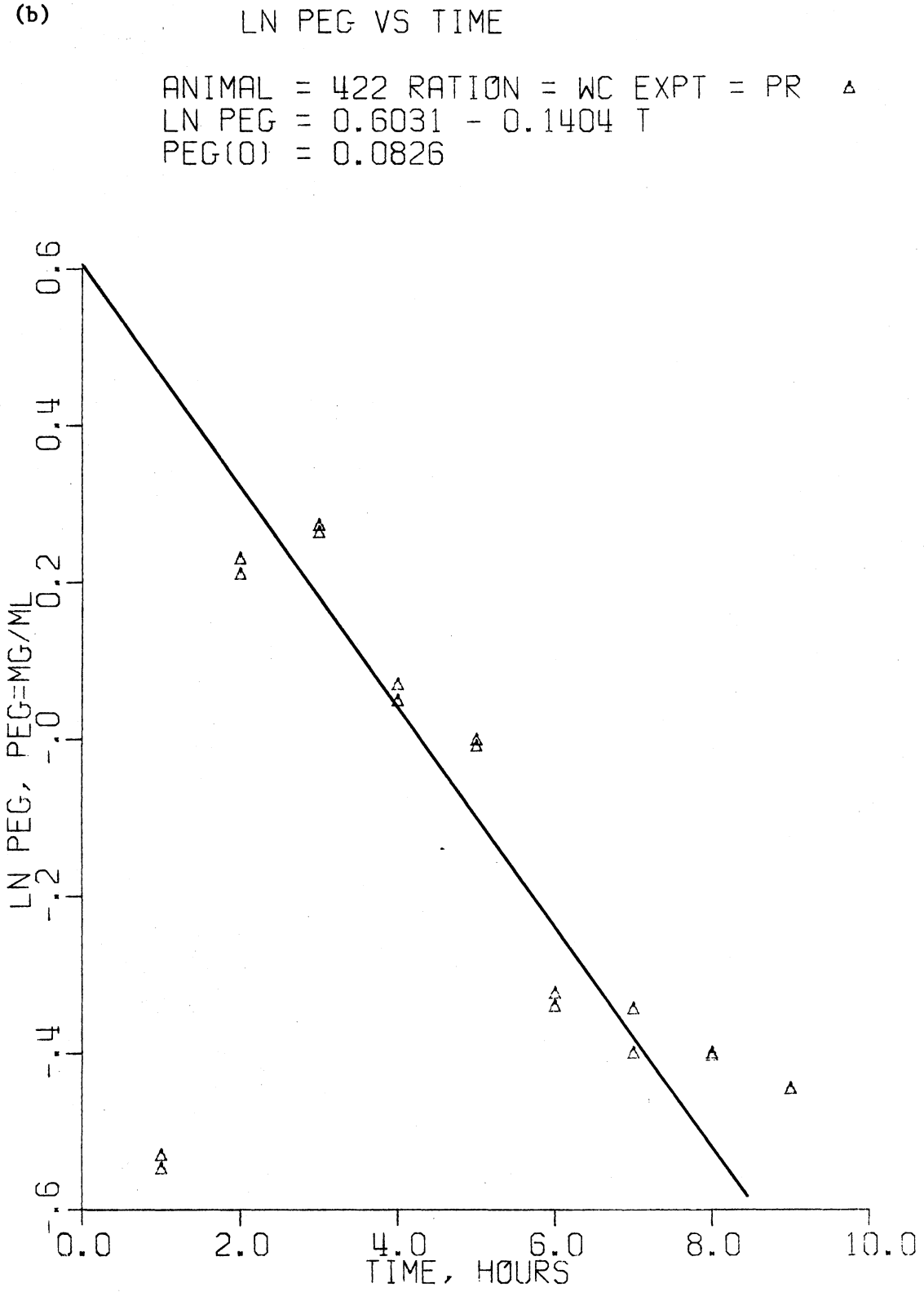


Figure 27. (Continued)

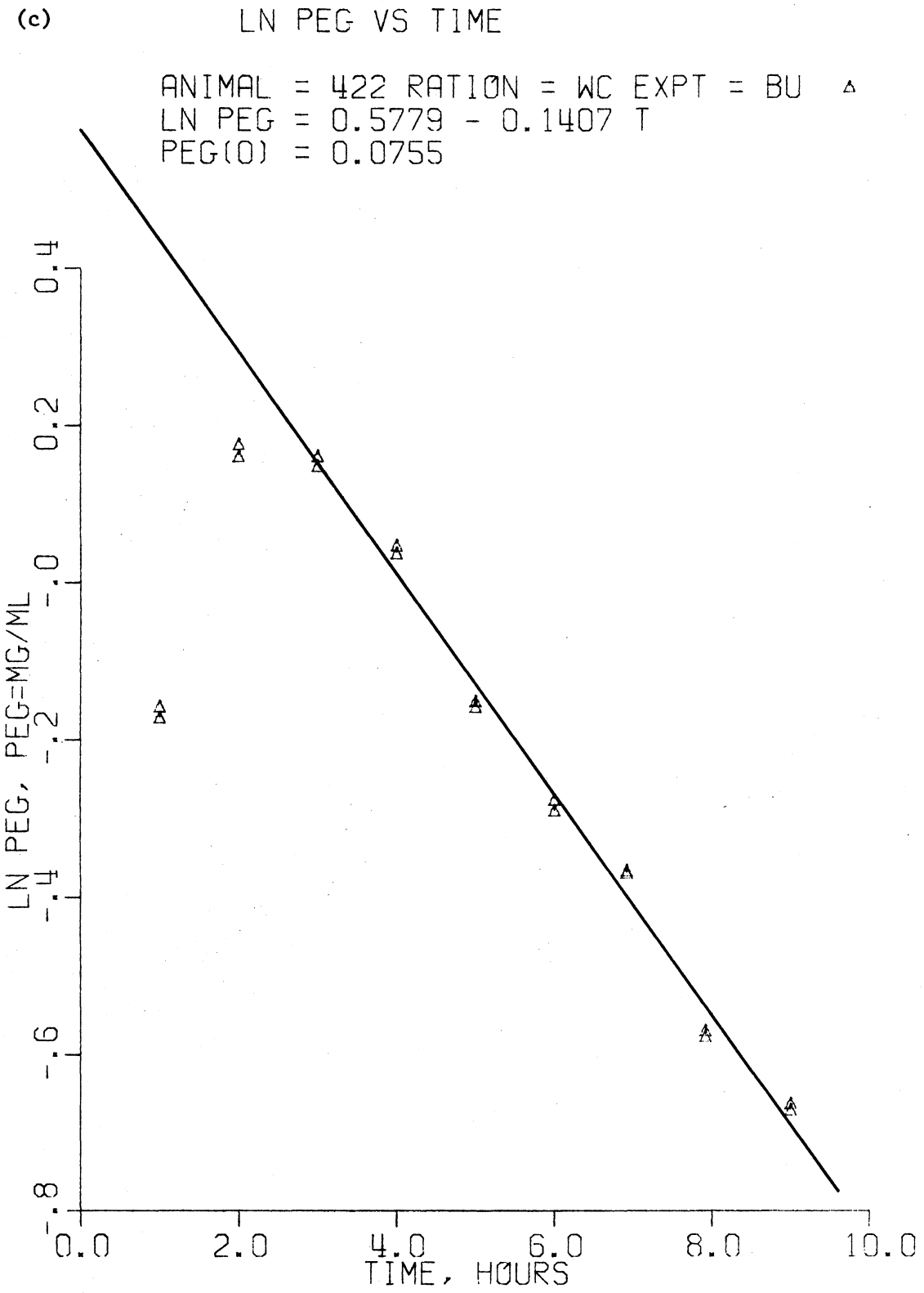


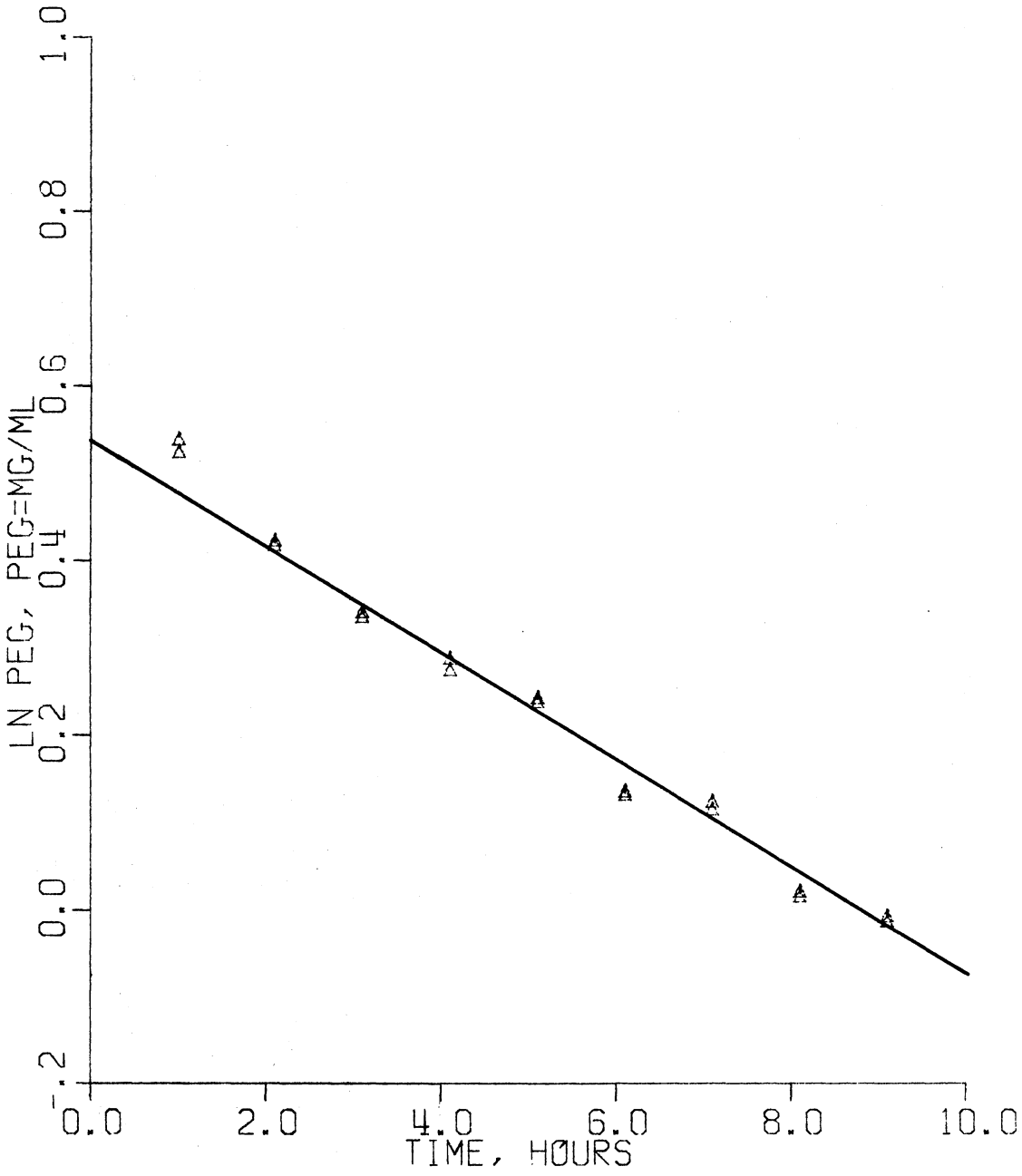
Figure 27. (Continued)

Figure 28. The Natural Logarithm of Rumen PEG Concentration versus Time for Animal 612 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)

LN PEG VS TIME

ANIMAL = 612 RATION = GC EXPT = AC
LN PEG = 0.5407 - 0.0619 T
PEG(0) = 0.0602



(b)

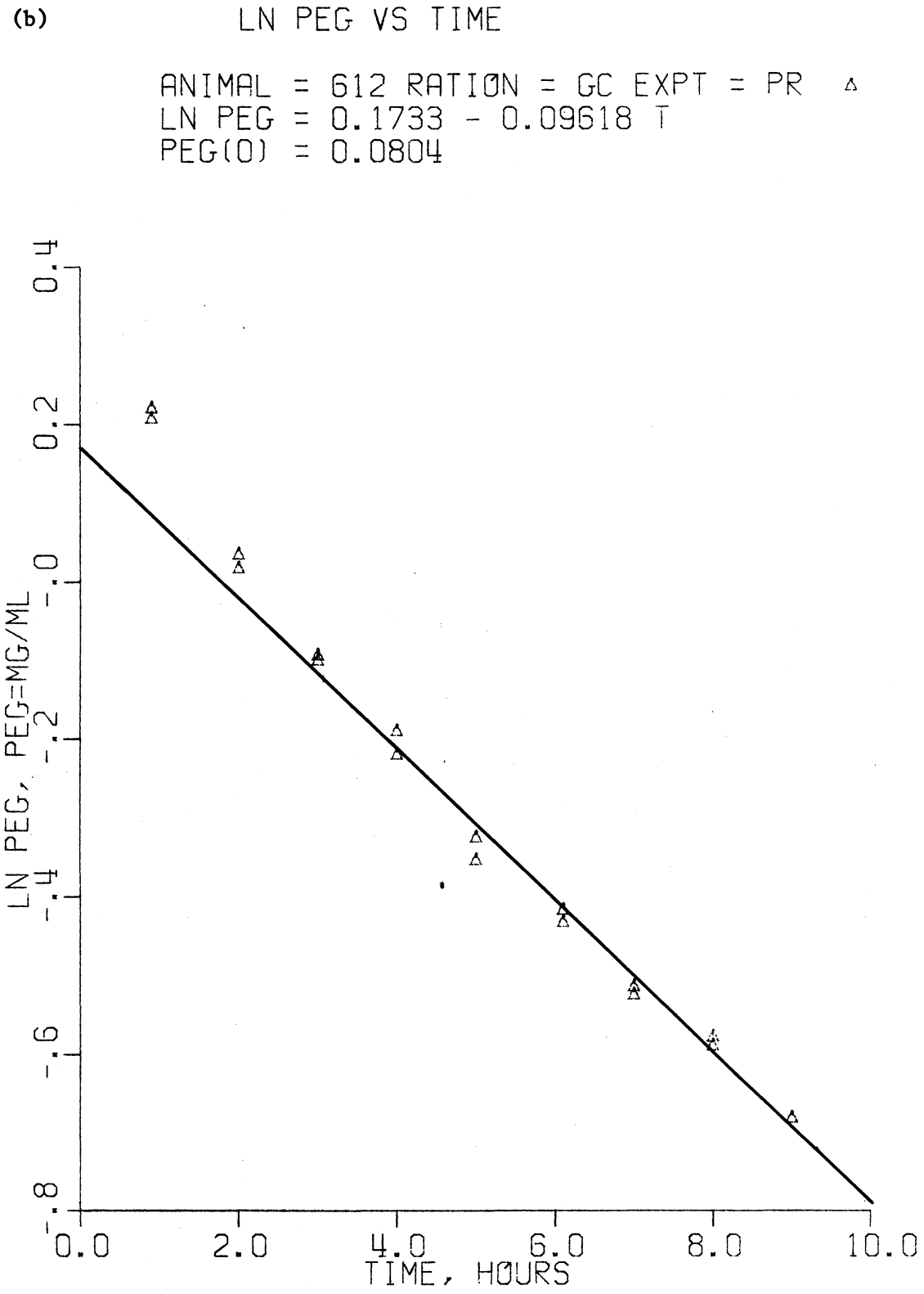


Figure 28. (Continued)

(c)

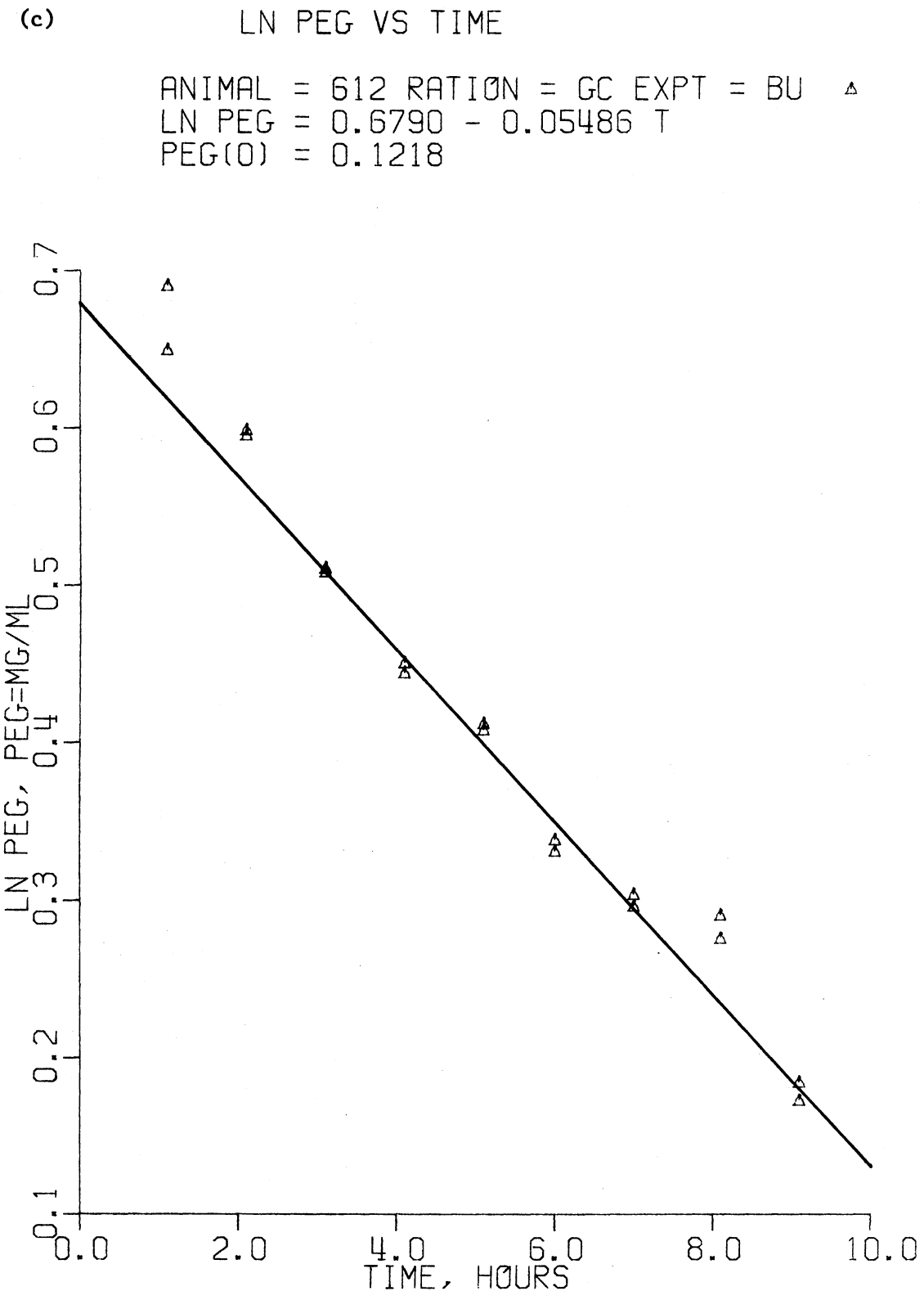


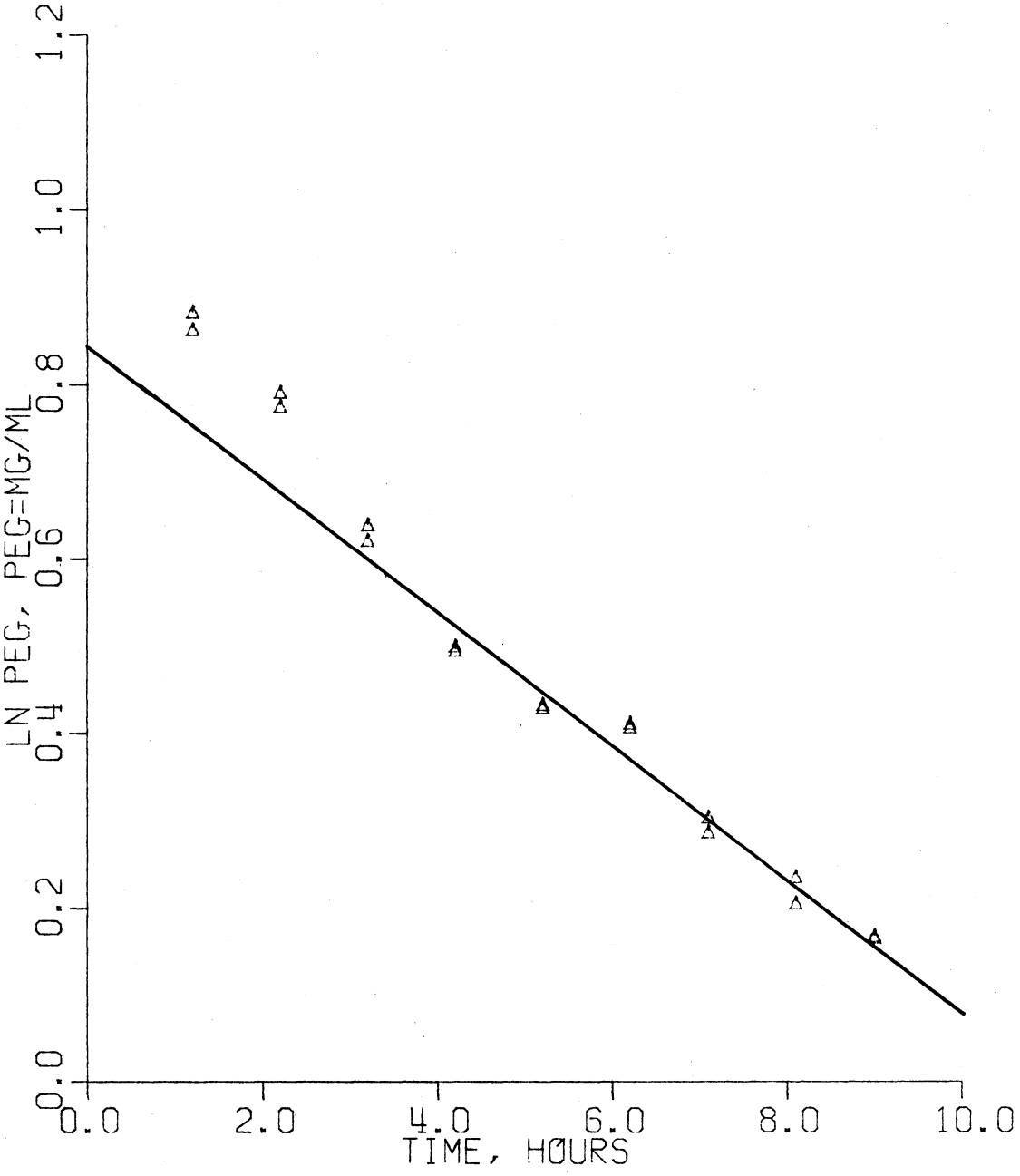
Figure 28. (Continued)

Figure 29. The Natural Logarithm of Rumen PEG Concentration versus Time for Animal 608 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)

LN PEG VS TIME

ANIMAL = 608 RATION = GC EXPT = AC △
LN PEG = 0.8446 - 0.07663 T
PEG(0) = 0.0857



(b)

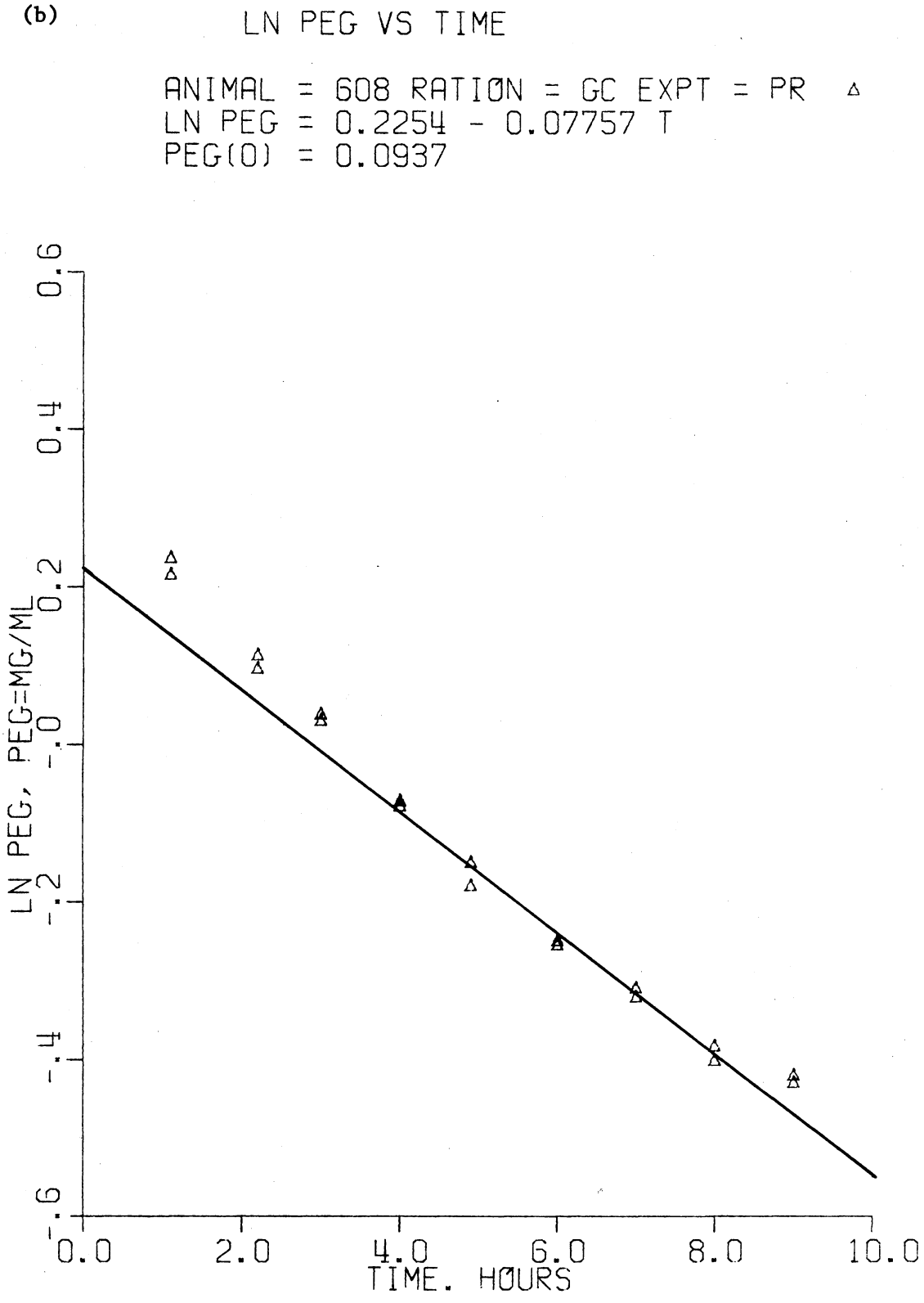


Figure 29. (Continued)

(c)

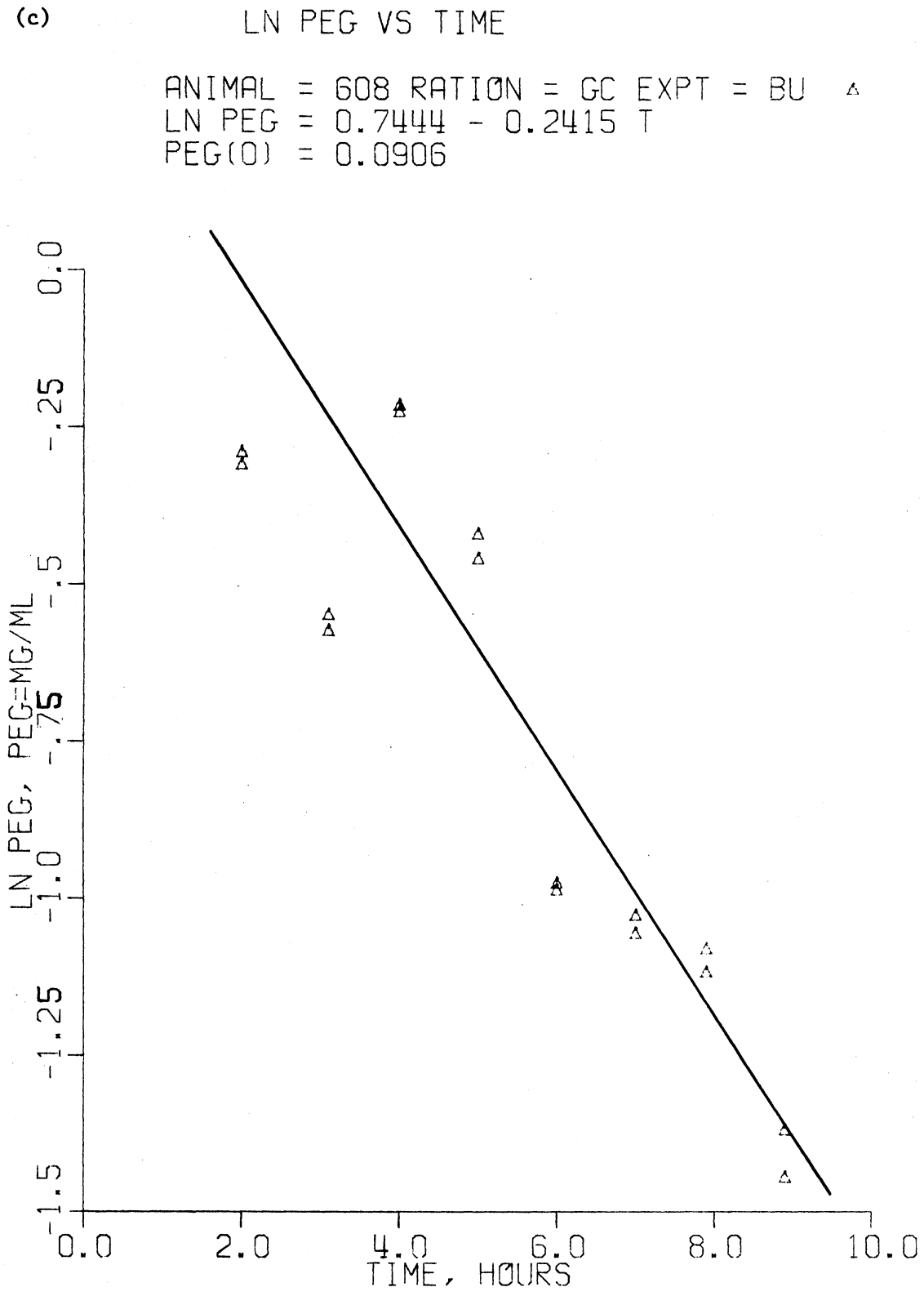


Figure 29. (Continued)

D is the dose of PEG at t=0 (50,000 mg).

The rumen liquid volume, turnover rate constant (dilution rate) and related parameters for each isotope infusion experiment are presented in Table XXVI. Animal 608 consuming the WC ration exhibited similar liquid volumes and turnover rate constants during the Pr and Bu infusion experiment, but these were markedly different than that obtained during the Ac infusion. Animal 422 had very consistent liquid volumes and dilution rates during all three infusions. Animal 612 was found to have similar liquid volumes and turnover rate constants during the Ac and Bu infusions, but these were somewhat higher during the Pr infusion. Rumen liquid volumes and turnover rate constants measured in Animal 608 while consuming the GC ration were quite variable. The liquid volumes were similar during the Ac and Bu infusions while dilution rates were similar during the Ac and Pr infusions. During the Bu infusion an abnormally high rumen dilution rate was observed, suggesting the possibility of an altered rumen fermentation. This was substantiated, as will be discussed later, by the proportions and concentrations of VFA found during this particular experiment.

Table XXVII summarizes the data presented in Table XXVI. Rumen volume appeared to be similar regardless of ration type; however, the steers consuming the WC ration tended to have higher ($P < .10$) liquid turnover rate constants (11.0 vs 7.4 %/hr). This difference may be related to the level of DMI, since the steers on WC ate more feed. Comparison of the DMI during each infusion experiment and the observed liquid dilution rate (Figure 30) seems to generally support this hypothesis. For example, Animal 608 consuming the whole corn

TABLE XXVI

RUMEN LIQUID VOLUMES, TURNOVER RATE CONSTANTS AND RELATED PARAMETERS

Animal	Diet	Expt	V^a (l)	$k_m{}^b$ (hr^{-1})	T^c (hr)	$T_{1/2}{}^d$ (hr)	$f_m{}^e$ (1/hr)	$f_m{}^e$ (ml/min)
608	WC	Ac	39.7	0.134	7.5	5.2	5.31	88.5
608	WC	Pr	25.1	0.054	18.7	12.9	1.35	22.4
608	WC	Bu	22.3	0.061	16.5	11.4	1.35	22.6
422	WC	Ac	28.2	0.128	7.8	5.4	3.61	60.1
422	WC	Pr	28.7	0.140	7.1	4.9	4.03	67.2
422	WC	Bu	29.3	0.141	7.1	4.9	4.12	68.7
612	GC	Ac	30.2	0.062	16.2	11.2	1.87	31.2
612	GC	Pr	45.1	0.096	10.4	7.2	4.34	72.3
612	GC	Bu	27.0	0.055	18.2	12.6	1.48	24.7
608	GC	Ac	22.3	0.077	13.1	9.0	1.71	28.5
608	GC	Pr	43.1	0.078	12.9	8.9	3.35	55.7
608	GC	Bu	24.8	0.242	4.1	2.9	5.99	99.8

[a]Rumen liquid volume.

[b]Liquid turnover rate constant.

[c]Liquid turnover time; $T_i = 1/k_{mi}$.

[d]Half-life of the liquid volume; $T_{1/2} = 0.693/k_m$.

[e]Rumen liquid outflow; $f_{mi} = k_{mi} V_i$.

TABLE XXVII

ANIMAL RATION PERIOD MEANS FOR RUMEN LIQUID VOLUMES, TURNOVER RATE CONSTANTS AND RELATED PARAMETERS

Period	n	V (l)	k_m (hr^{-1})	T (hr)	$T_{\frac{1}{2}}$ (hr)	F_m (l/hr)	F_m (ml/min)
608-WC	3	29.0	0.083	14.2	9.8	2.67	44.5
422-WC	3	28.7	0.136	7.3	5.1	3.92	65.3
612-GC	3	34.1	0.071	14.9	10.3	2.56	42.7
608-GC	3	30.1	0.132	10.0	6.9	3.68	61.3
608-GC[a]	2	32.7	0.078	13.0	9.0	2.53	42.1
WC	6	28.9	0.110[d]	10.8	7.5	3.30	54.9
GC	6	32.1	0.102	12.5	8.6	3.12	52.0
GC[a]	5	33.5	0.074[e]	14.2	9.8	2.55	42.5
WC SE[b]	6	3.28	0.024	2.10	1.44	0.69	11.57
GC SE[c]	6	3.28	0.013	1.82	1.26	0.59	9.91
GC SE[c]	5	3.59	0.014	2.00	1.38	0.65	10.85

[a]The 608-GC-Bu infusion date was excluded.

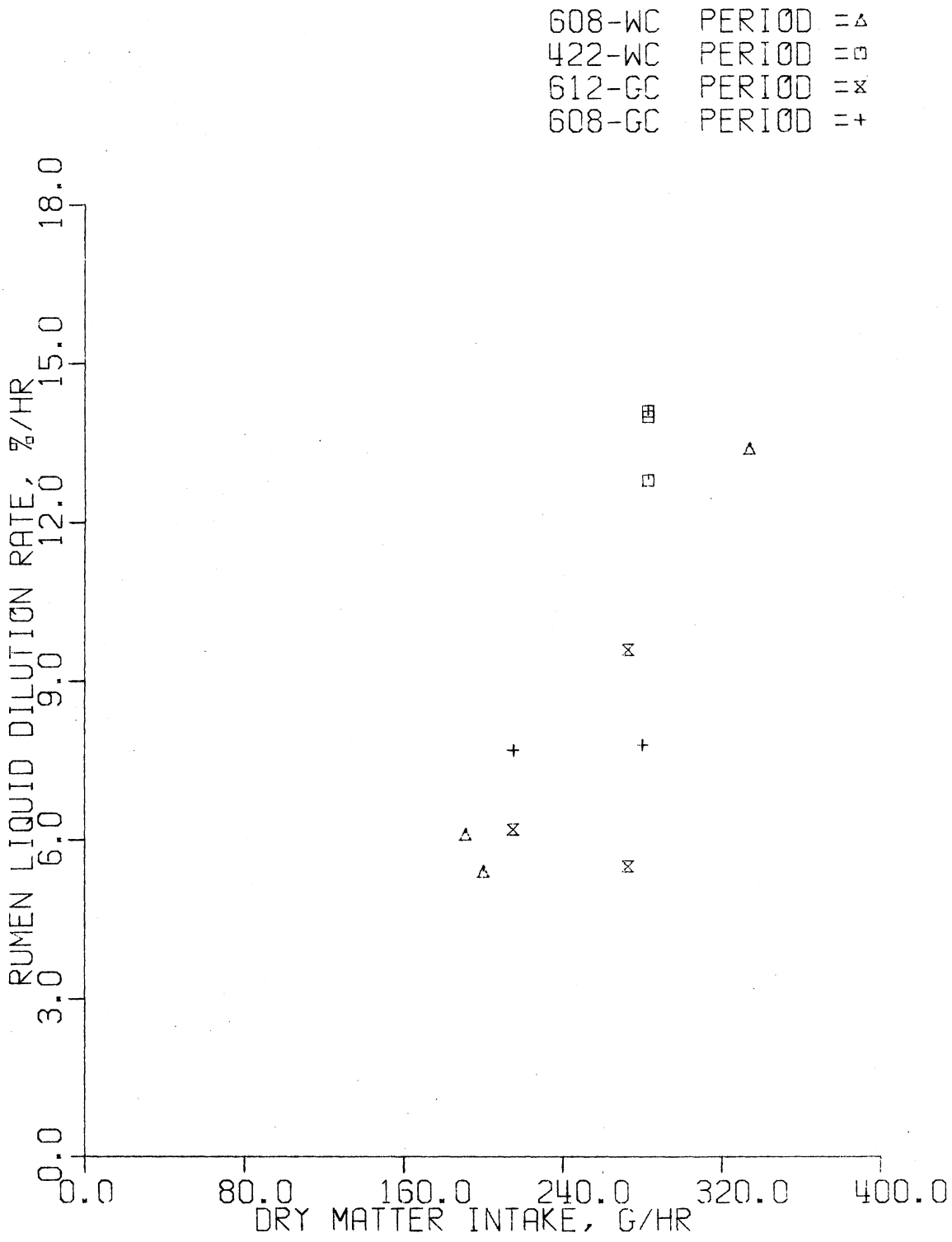
[b]Standard error of the mean.

[c]Standard error of the mean (excluding 608-GC-Bu data).

[d,e]Tended to be different ($P < .10$).

Figure 30. Relationship Between Rumen Liquid Dilution Rate and Dry Matter Intake.

RUMEN LIQUID DILUTION RATE VS DRY MATTER INTAKE



diet had a higher DMI and a higher dilution rate during the Ac infusion than was observed on the other two infusions in this period (334 g/hr and 13.4%/hr versus 196 g/hr and 5.8%/hr, respectively). An explanation for the very high dilution rate observed in Animal 608 consuming the ground corn diet during the Bu infusion is not readily apparent.

pH. The pH of the rumen during each isotope infusion is presented in Figure 31 (a-d). The mean pH for each infusion experiment is given in Table XXVIII while the average pH for each ration and animal-ration period is shown in Table XXIX.

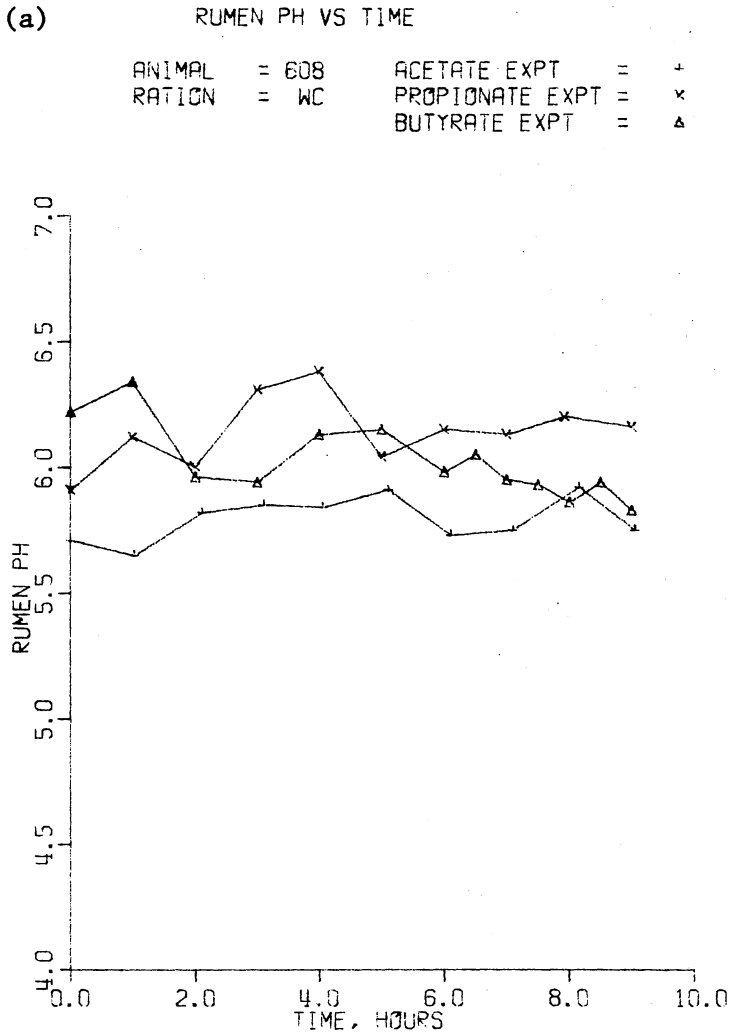
Ruminal pH remained fairly constant within each infusion suggesting the presence of steady-state conditions. This observation supports the assumption that hourly feeding reduces the diurnal variation in several rumen parameters suggesting that a more constant fermentation is occurring under these conditions. Rumen pH was relatively consistent within each animal-ration period; however, differences between periods (Table XXIX) appeared related to animal and possibly ration effects.

Animal 608 maintained a higher pH on WC and GC than Animal 422 and Animal 612, respectively. Genetic variation reflected in saliva flow and/or buffering capacity could possibly have contributed to this effect. However, in both cases Animal 608 had a greater feed intake than the other animal, suggesting the possibility of increased saliva flow and, subsequently, an increased rumen pH due to a higher level of feed intake (Putnam et al., 1966b). However, rumen liquid turnover (Table XXVII) was not greater.

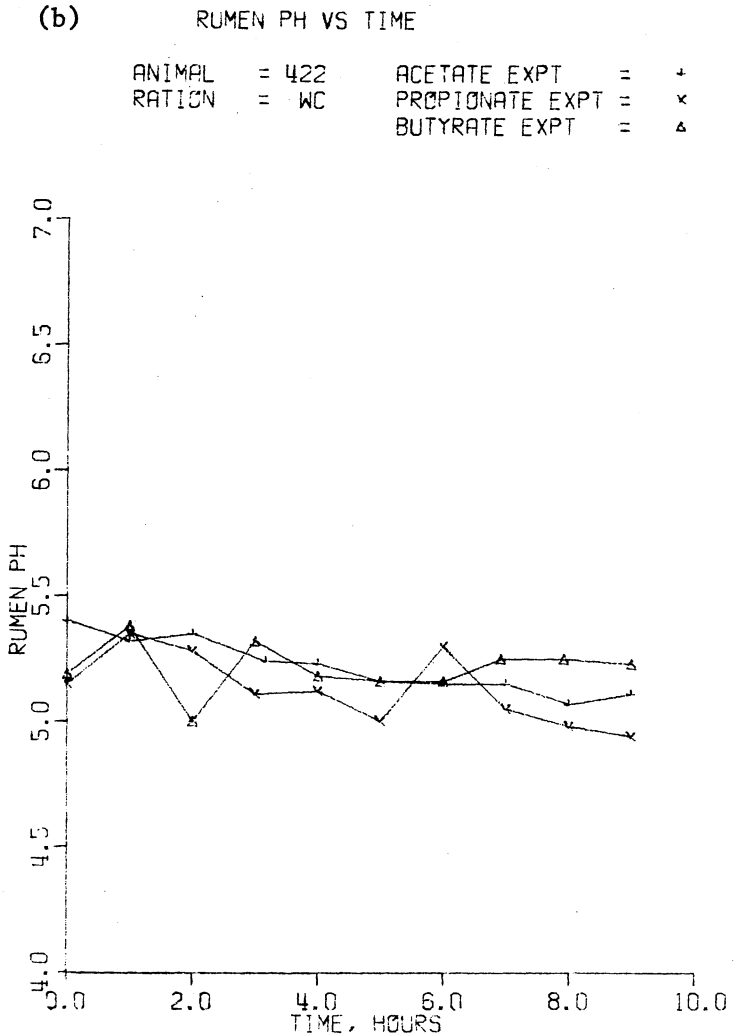
Animal 608 had a lower rumen pH when consuming the GC ration as

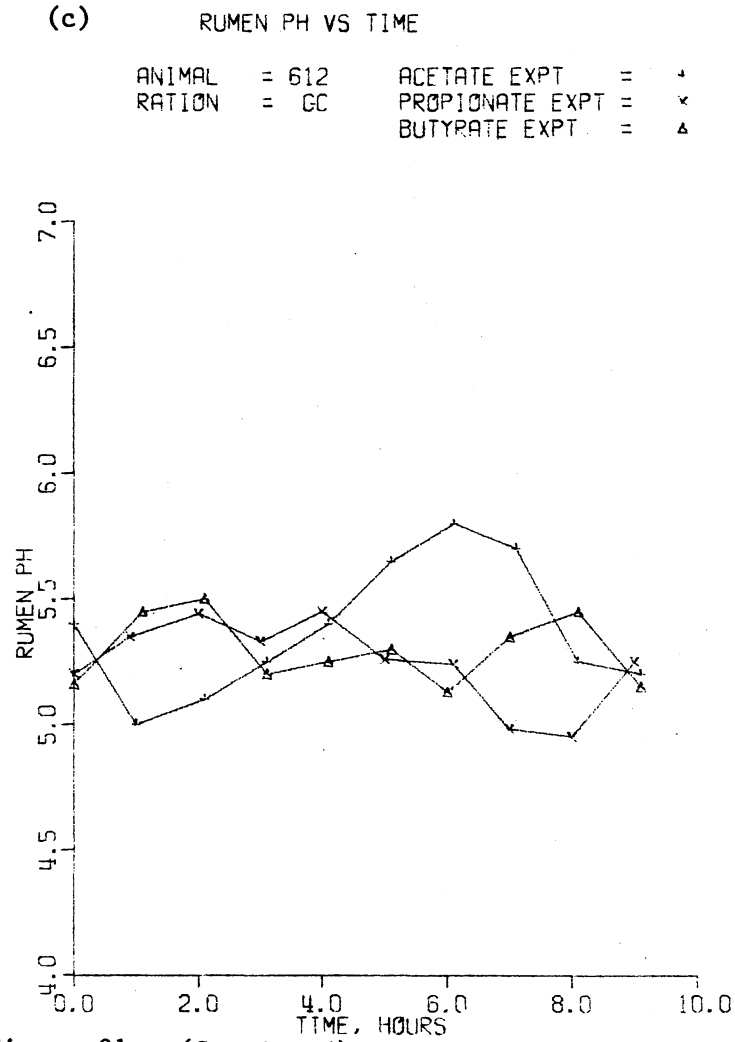
Figure 31. Rumen pH versus Time for Each Infusion Experiment.
(a) Animal 608, Ration- WC; (b) Animal 422, Ration- WC;
(c) Animal 612, Ration- GC; (d) Animal 608, Ration- GC.

(a)


RUMEN PH VS TIME
ANIMAL = 608
RATION = WC
ACETATE EXPT = +
PROPIONATE EXPT = ×
BUTYRATE EXPT = Δ
RUMEN PH
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)


RUMEN PH VS TIME
ANIMAL = 422
RATION = WC
ACETATE EXPT = +
PROPIONATE EXPT = ×
BUTYRATE EXPT = Δ
RUMEN PH
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS



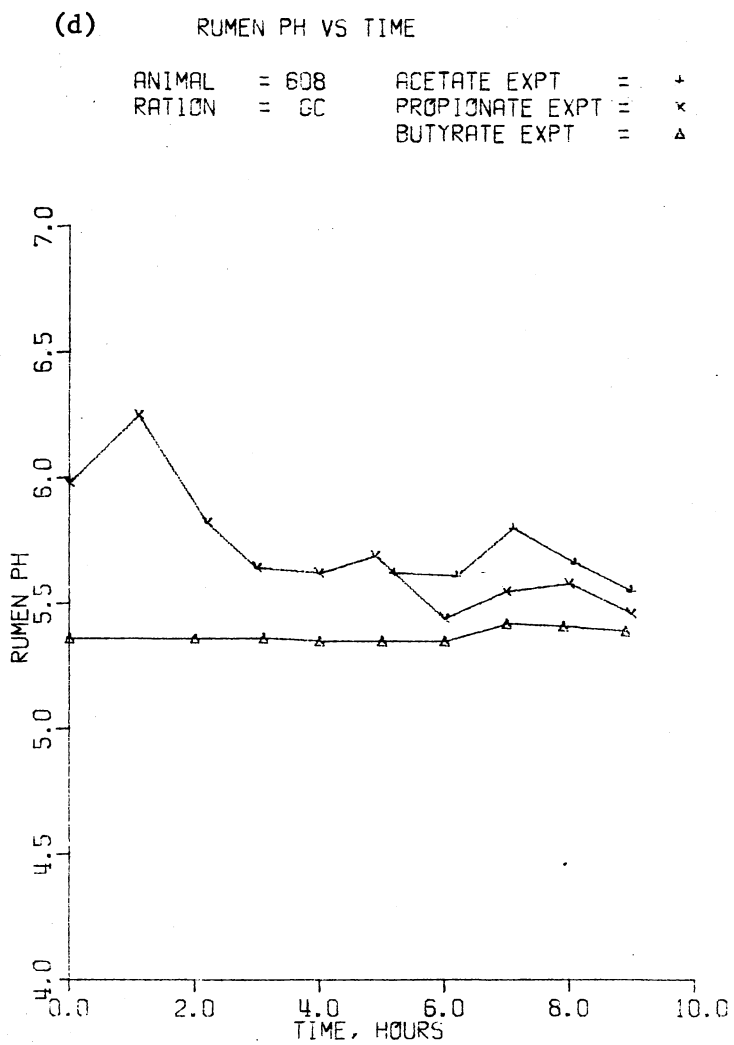


Figure 31. (Continued)

TABLE XXVIII

MEAN RUMINAL pH FOR EACH INFUSION EXPERIMENT

Animal	Diet	Expt	n	mean pH
608	WC	Ac	10	5.78
		Pr	10	6.12
		Bu	13	6.00
422	WC	Ac	10	5.21
		Pr	10	5.11
		Bu	10	5.20
612	GC	Ac	10	5.31
		Pr	10	5.21
		Bu	10	5.28
608	GC	Ac	5	5.64
		Pr	10	5.65
		Bu	9	5.39

TABLE XXIX

MEAN RUMINAL pH FOR EACH ANIMAL-RATION PERIOD

Period	n	pH
608-WC	3	5.94
422-WC	3	5.17
Mean	6	5.40
612-GC	3	5.26
608-GC	3	5.53
Mean	6	5.37

compared to when the WC diet was fed. This difference may be related to the higher level of feed intake on the WC ration or to an elevated fermentation rate on the GC diet (Vance et al., 1970; White et al., 1975b). Rumen pH on the whole and ground corn rations was not different.

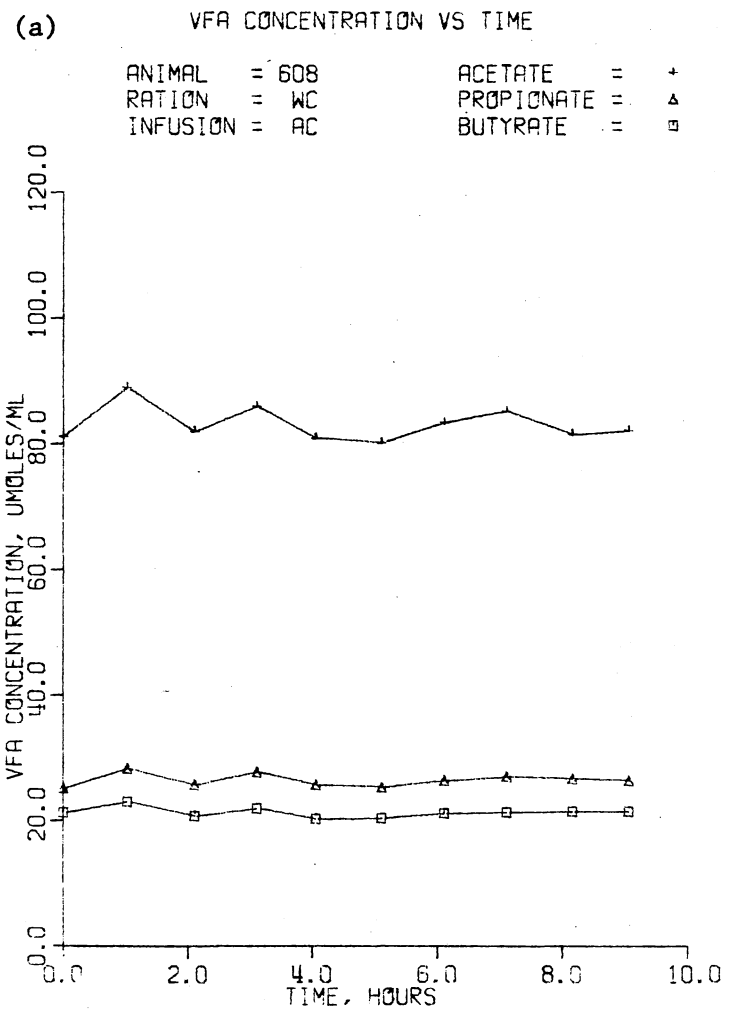
VFA Concentration. Volatile fatty acid concentrations during each infusion experiment are presented in Figures 32-36. The means for each infusion are given in Table XXX, while the period means are presented in Table XXXI.

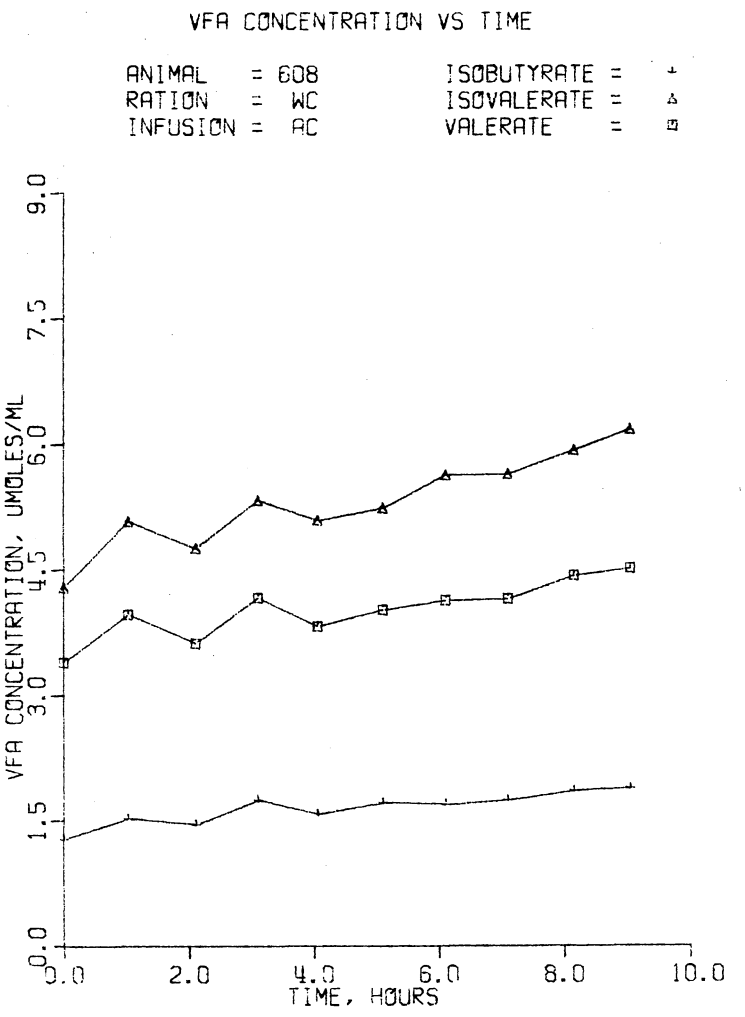
In general, VFA concentration remained fairly constant over a given infusion experiment suggesting that the fermentation approached steady-state conditions. Coefficients of variation were usually within the range of 5 to 15% (Table XXX); however, some values were > 15%, especially those associated with the iso- and straight chain (C > 4) VFA's. This is probably due to the lower level of precision with which these acids can be quantitatively measured, since they are less concentrated than the major VFA's (acetic, propionic and butyric acids). This, however, does not preclude the possibility that these minor VFA's are more sensitive to fluctuations in the rumen fermentation.

The VFA pattern of Animal 608 was markedly different than that seen in Animal 422 when both were consuming the WC diet. Animal 608 had a fermentation pattern (high Ac/Pr) which resembled that typically observed in animals consuming forage diets, while Animal 422 exhibited a more 'normal' (low Ac/Pr) VFA pattern typical of steers fed a high-concentrate ration. Furthermore, Animal 422 had a higher ($P < .05$)

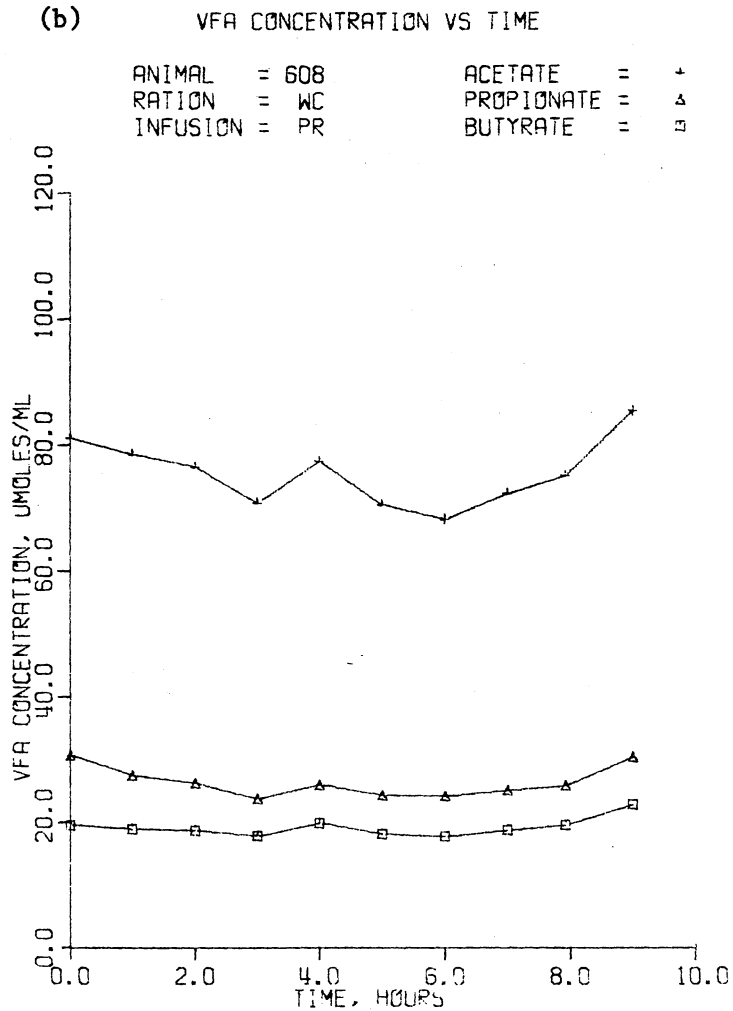
Figure 32. Ruminal Volatile Fatty Acid Concentration versus Time for Animal 608 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)


VFA CONCENTRATION VS TIME
ANIMAL = 608
RATION = WC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA CONCENTRATION, UMOLES/ML
0.0
20.0
40.0
60.0
80.0
100.0
120.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


VFA CONCENTRATION VS TIME
ANIMAL = 608
RATION = WC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA CONCENTRATION, UMOLES/ML
0.0
1.5
3.0
4.5
6.0
7.5
9.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



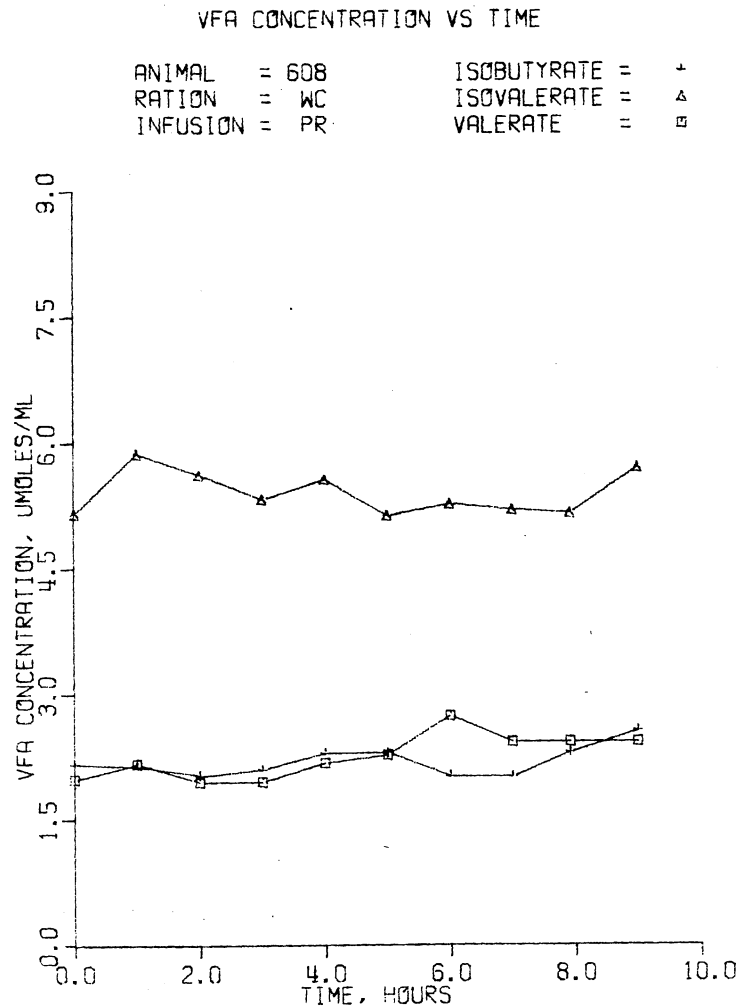
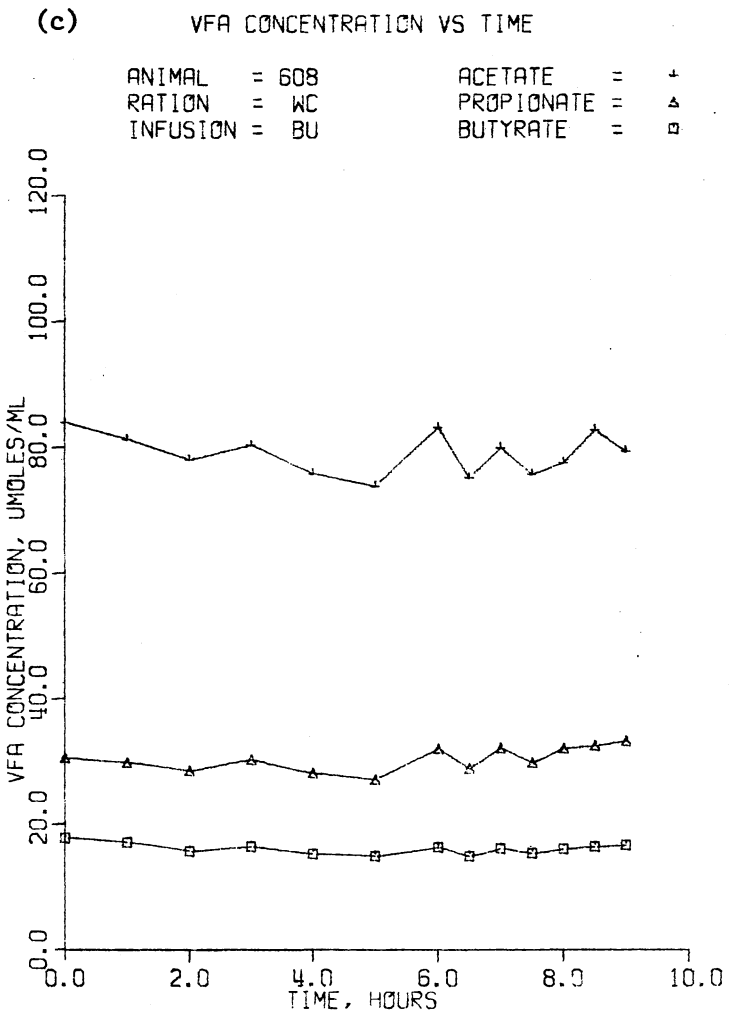


Figure 32. (Continued)

(c)



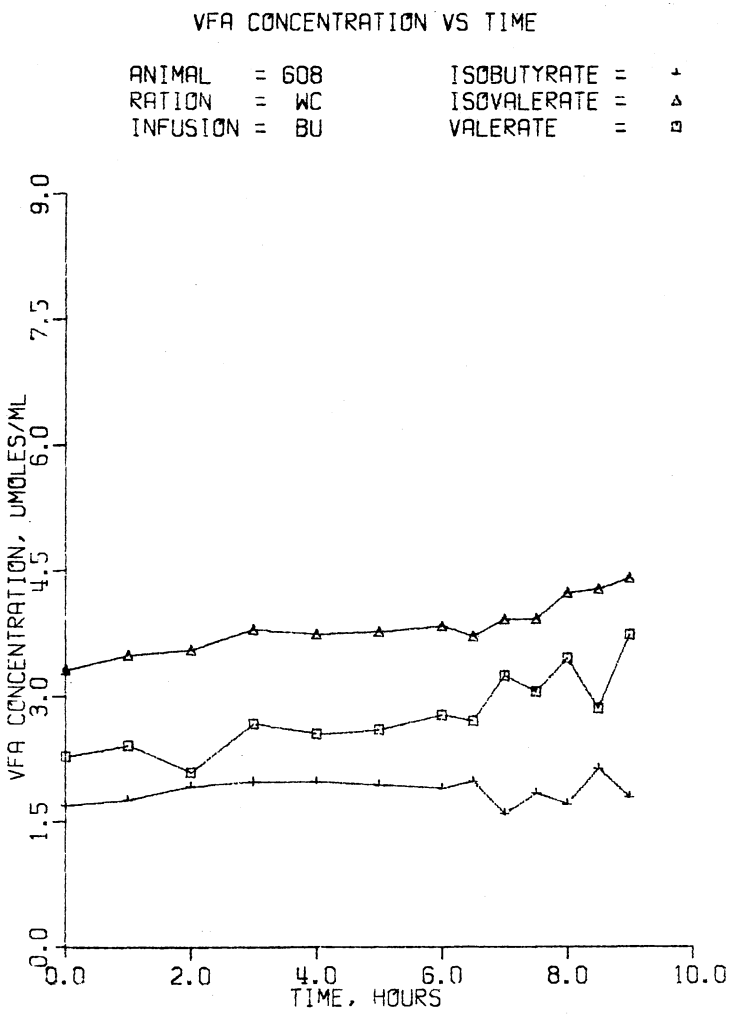
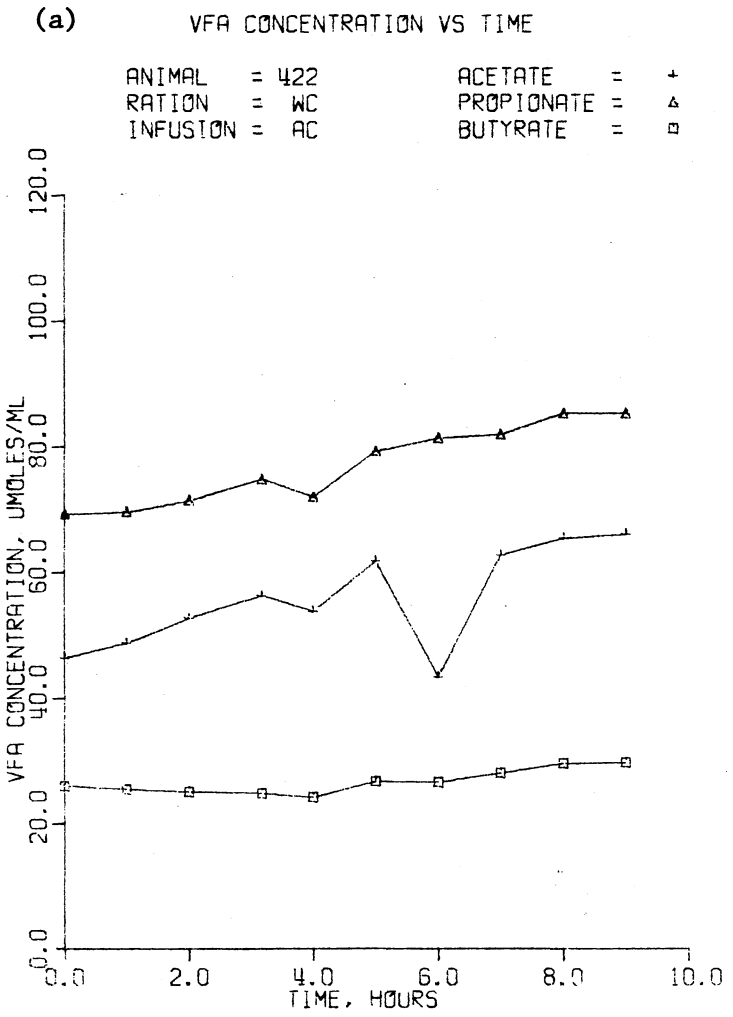
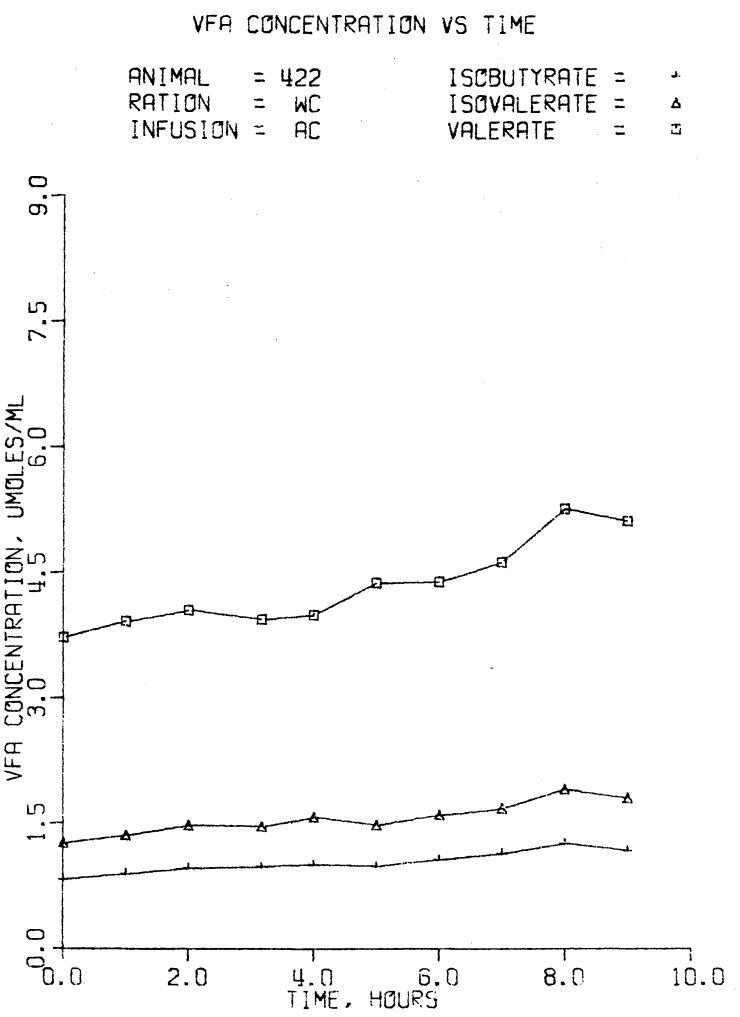


Figure 32. (Continued)

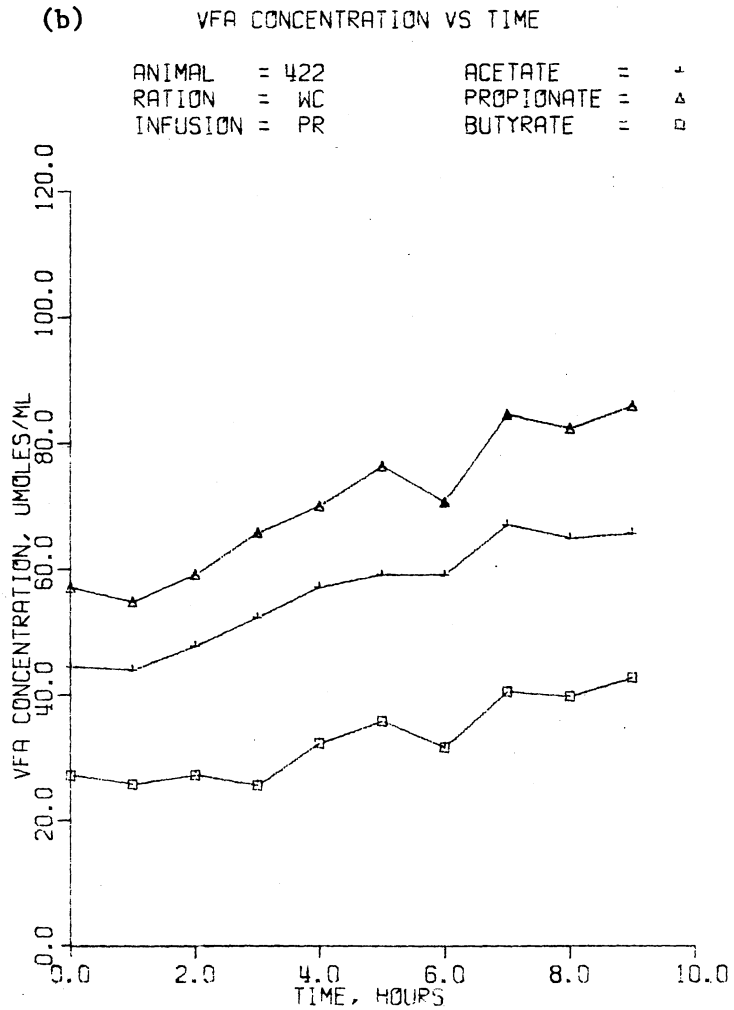
Figure 33. Ruminal Volatile Fatty Acid Concentration versus Time for Animal 422 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)


VFA CONCENTRATION VS TIME
ANIMAL = 422
RATION = WC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA CONCENTRATION, UMOLES/ML
0.0
20.0
40.0
60.0
80.0
100.0
120.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


VFA CONCENTRATION VS TIME
ANIMAL = 422
RATION = WC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA CONCENTRATION, UMOLES/ML
0.0
1.5
3.0
4.5
6.0
7.5
9.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



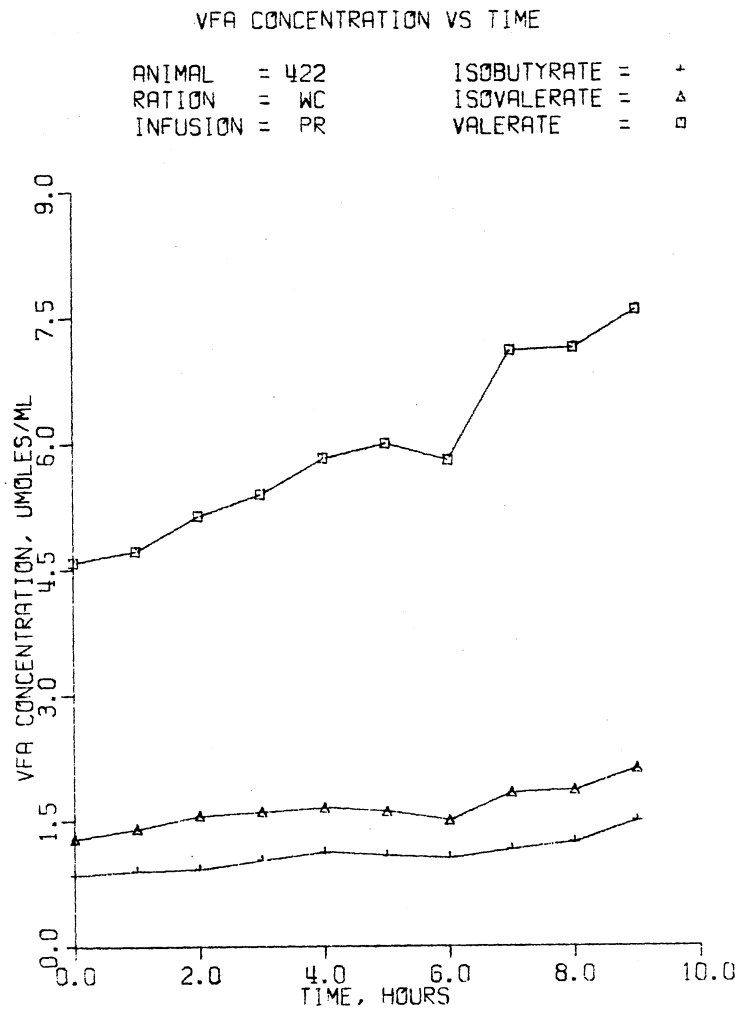
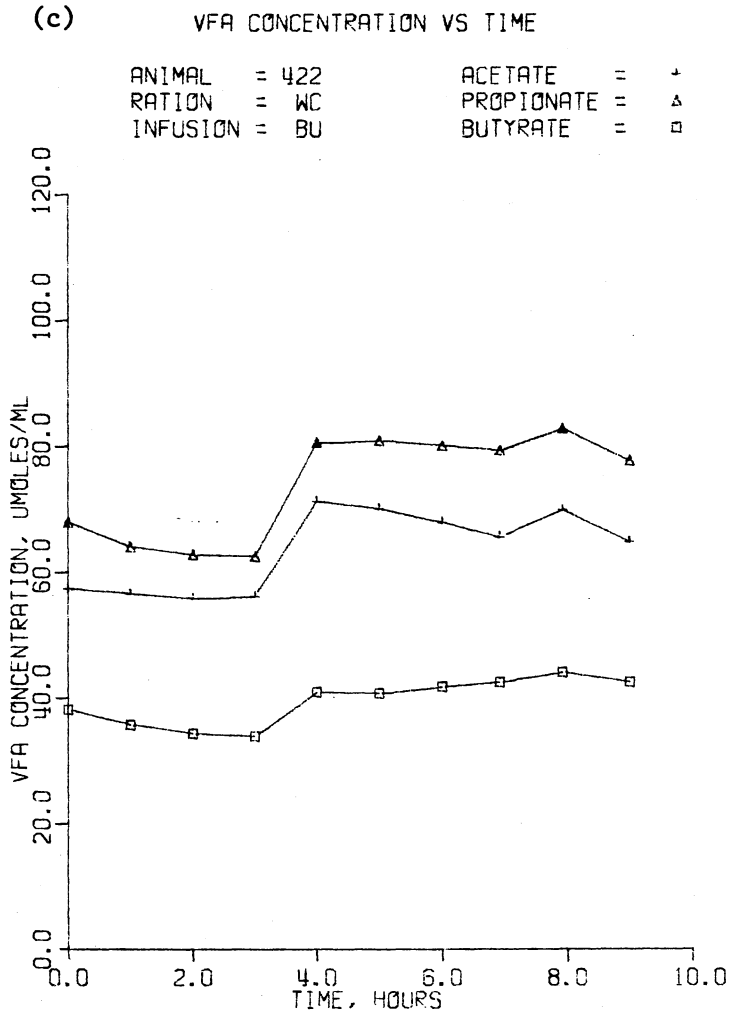


Figure 33. (Continued)

(c)



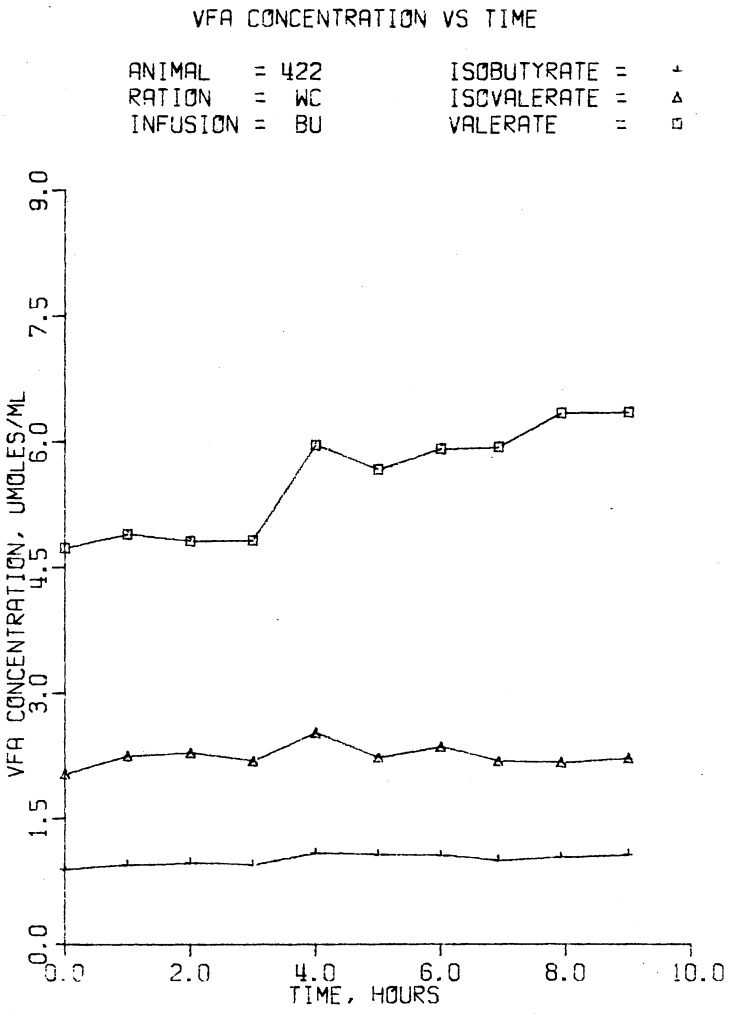
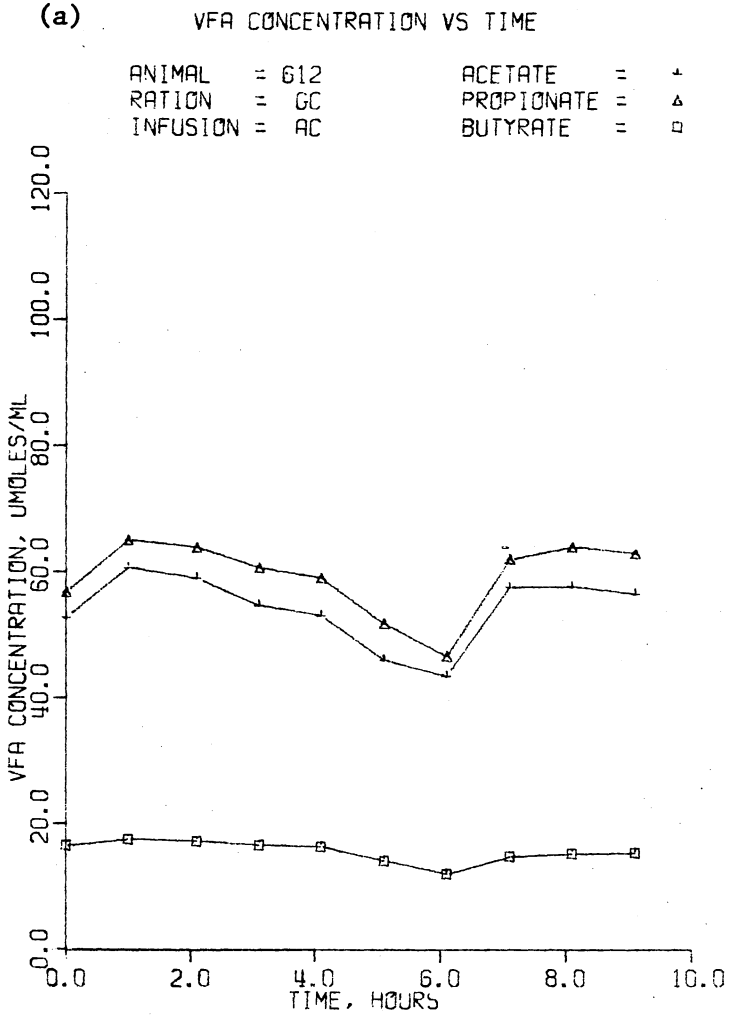
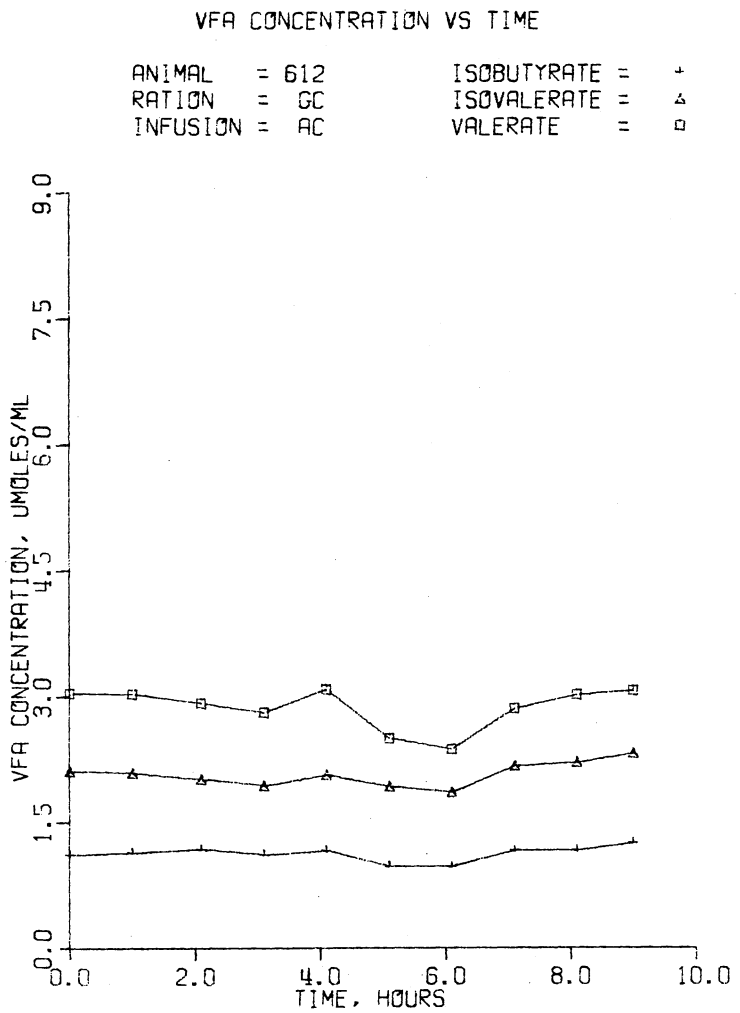


Figure 33. (Continued)

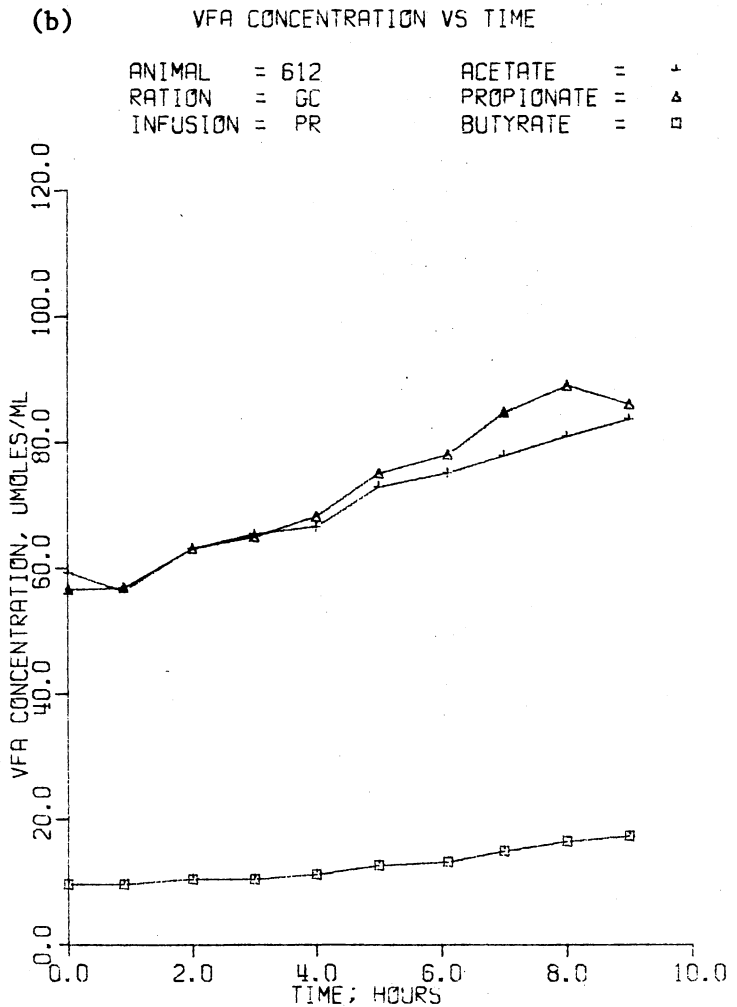
Figure 34. Ruminal Volatile Fatty Acid Concentration versus Time for Animal 612 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)


VFA CONCENTRATION VS TIME
ANIMAL = 612
RATION = GC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA CONCENTRATION, UMOLES/ML
0.0
20.0
40.0
60.0
80.0
100.0
120.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


VFA CONCENTRATION VS TIME
ANIMAL = 612
RATION = GC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA CONCENTRATION, UMOLES/ML
0.0
1.5
3.0
4.5
6.0
7.5
9.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



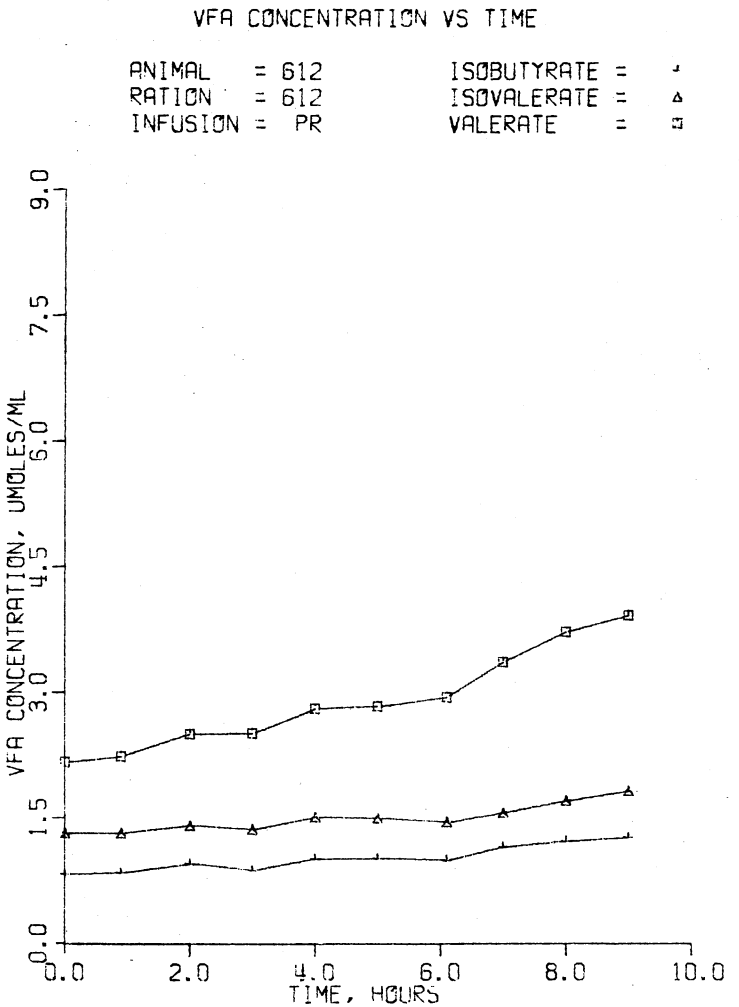
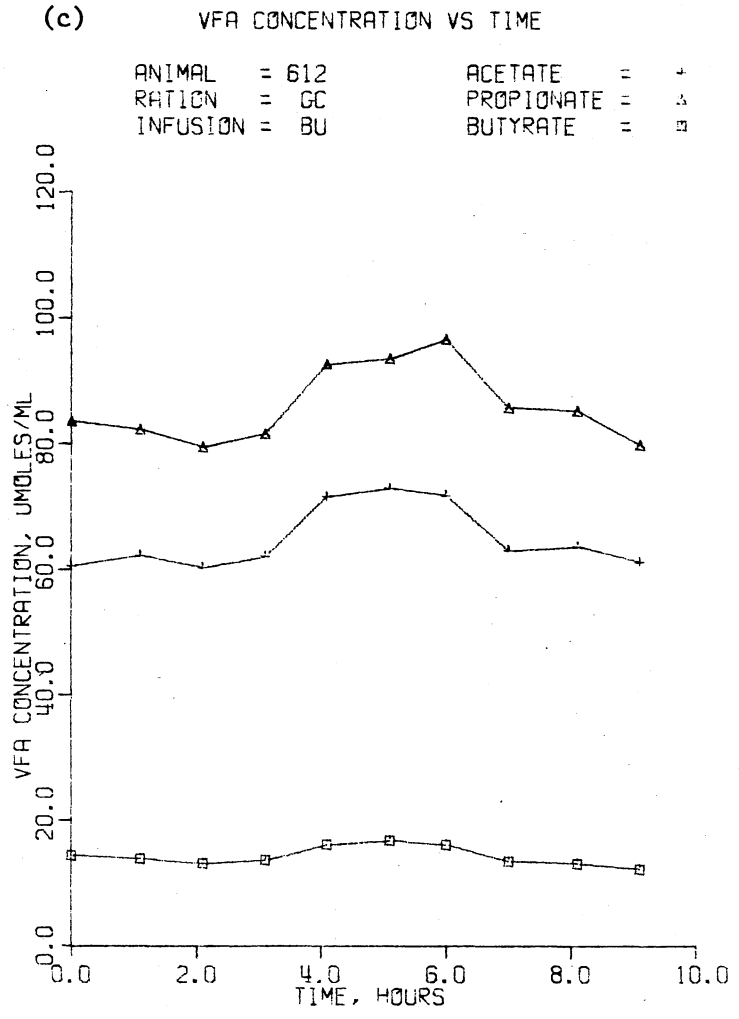


Figure 34. (Continued)

(c)



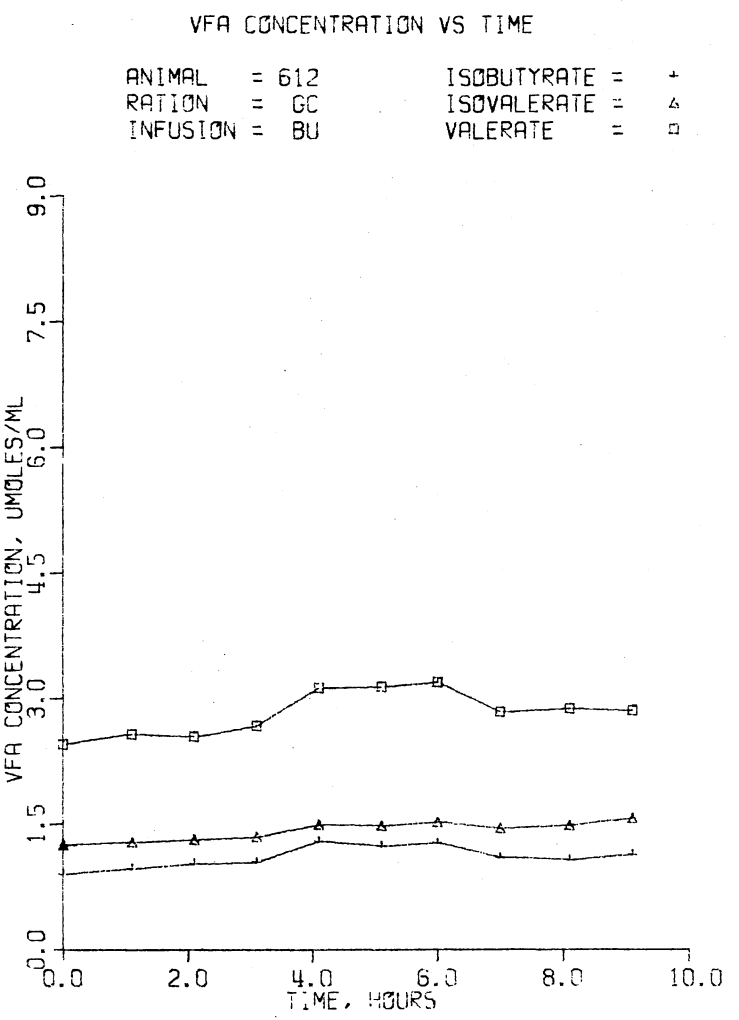
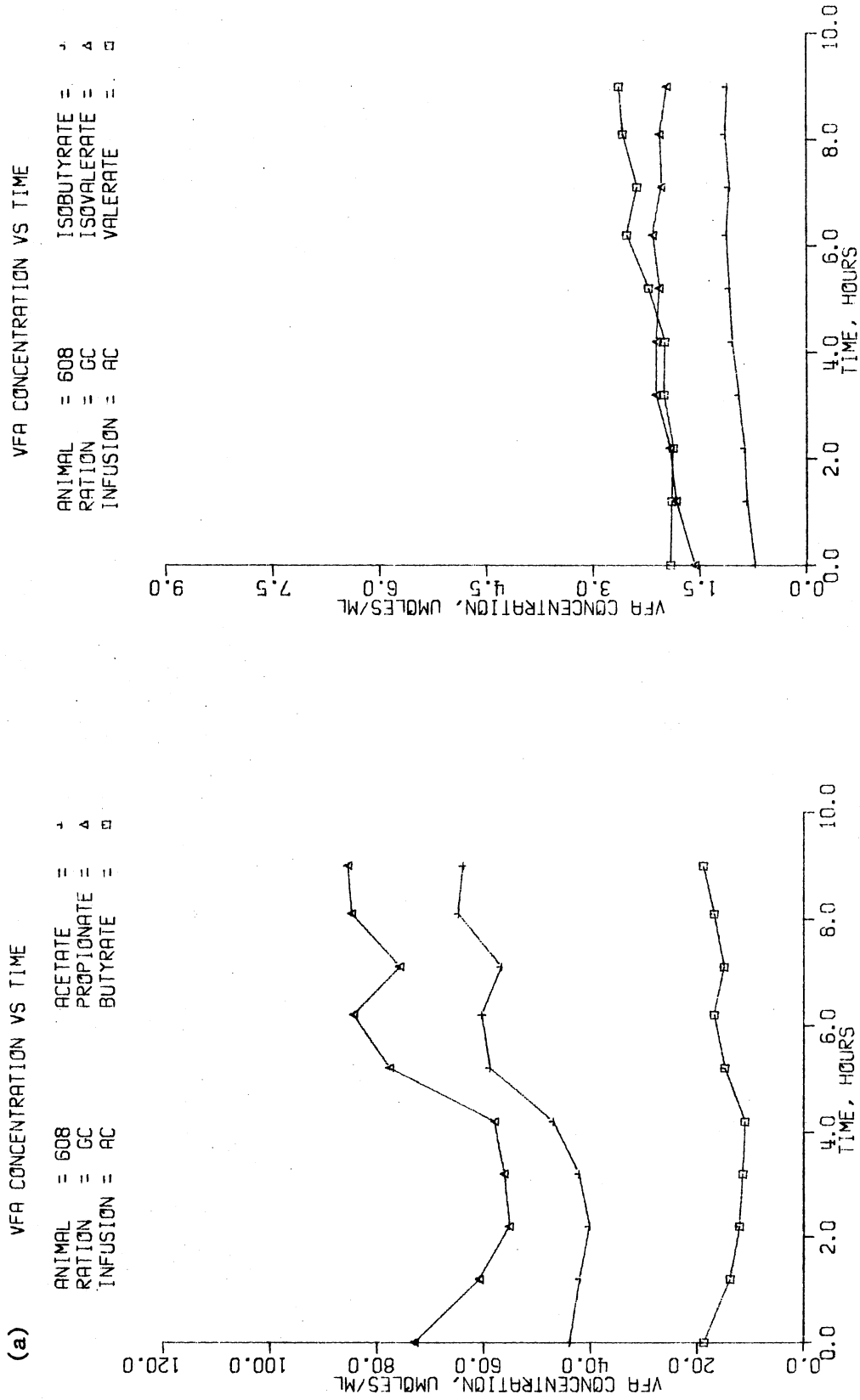
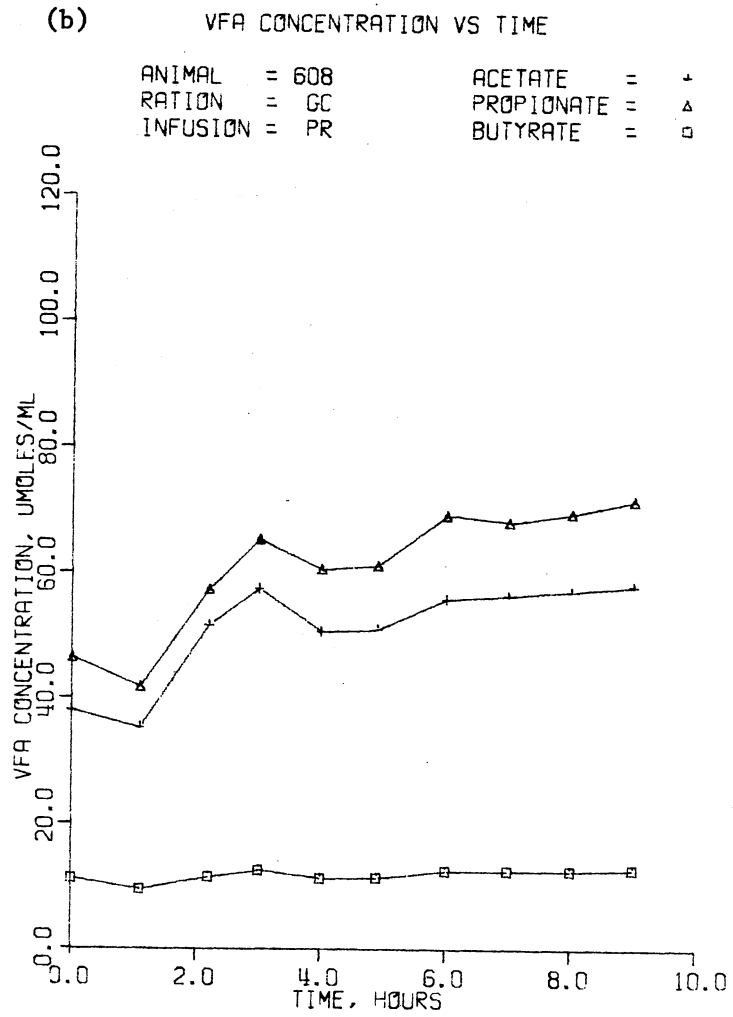


Figure 34. (Continued)

Figure 35. Ruminal Volatile Fatty Acid Concentration versus Time for Animal 608 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.


(a)
VFA CONCENTRATION VS TIME
ANIMAL = 608
RATION = GC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA CONCENTRATION, UMOLES/ML
0.0
20.0
40.0
60.0
80.0
100.0
120.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS
VFA CONCENTRATION VS TIME
ANIMAL = 608
RATION = GC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA CONCENTRATION, UMOLES/ML
0.0
1.5
3.0
4.5
6.0
7.5
9.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



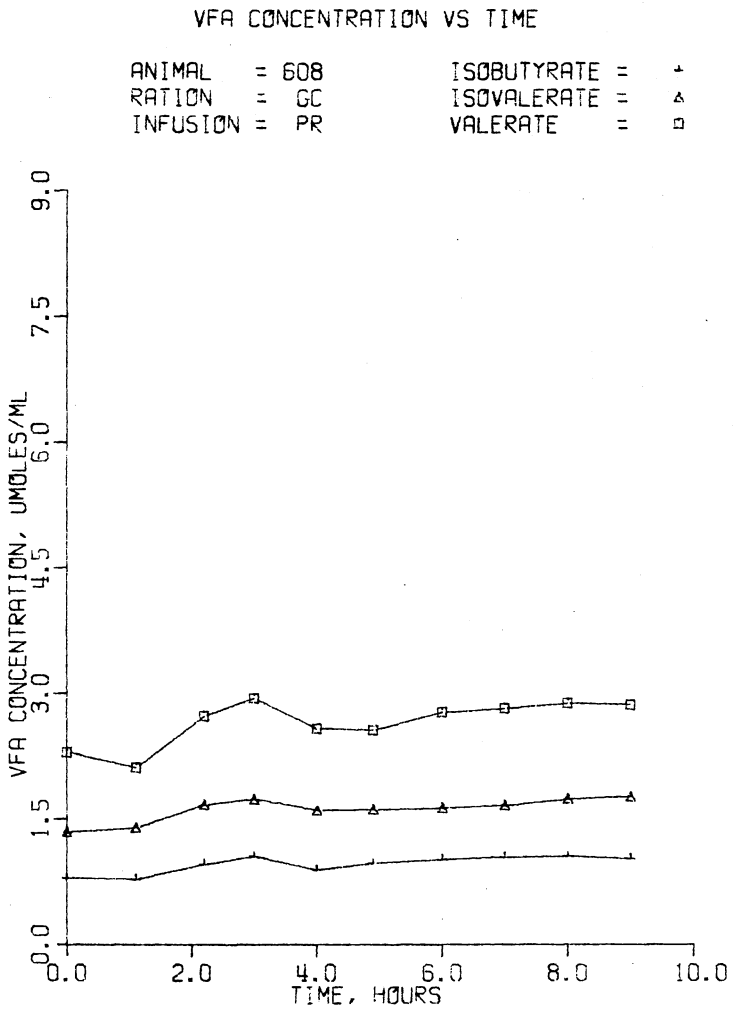
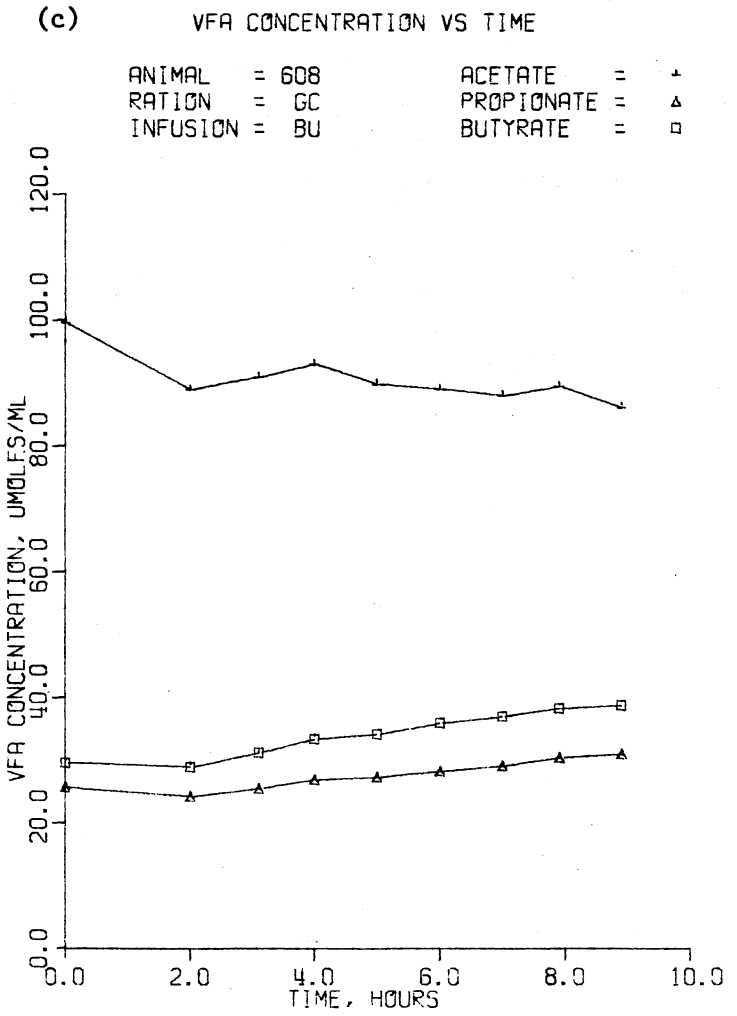


Figure 35. (Continued)



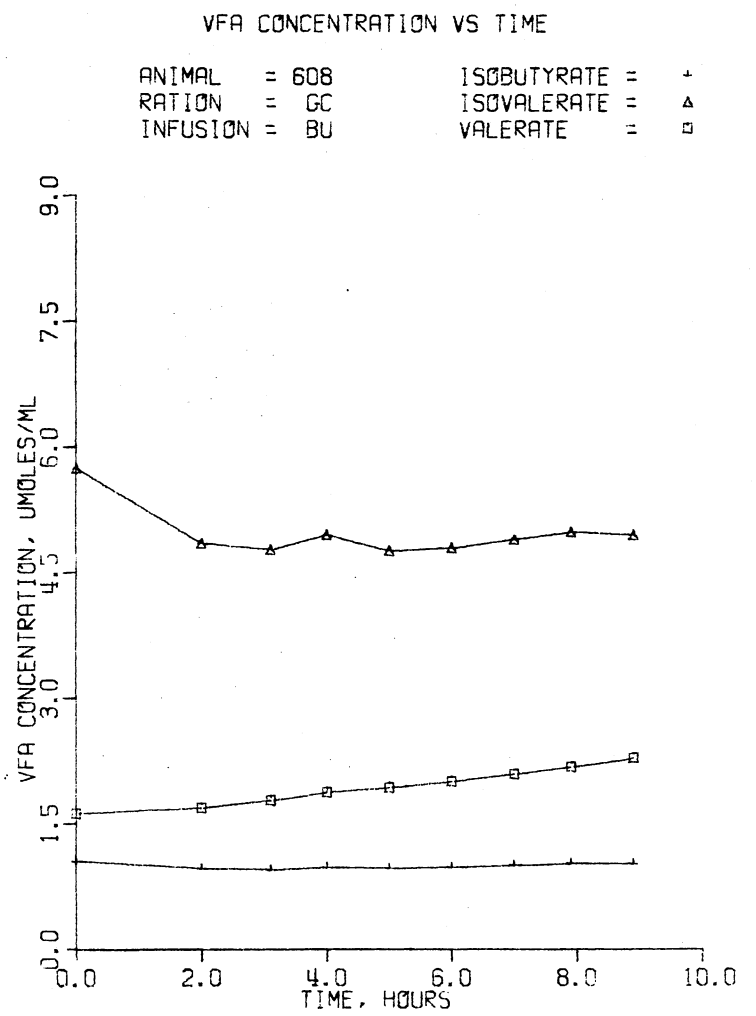


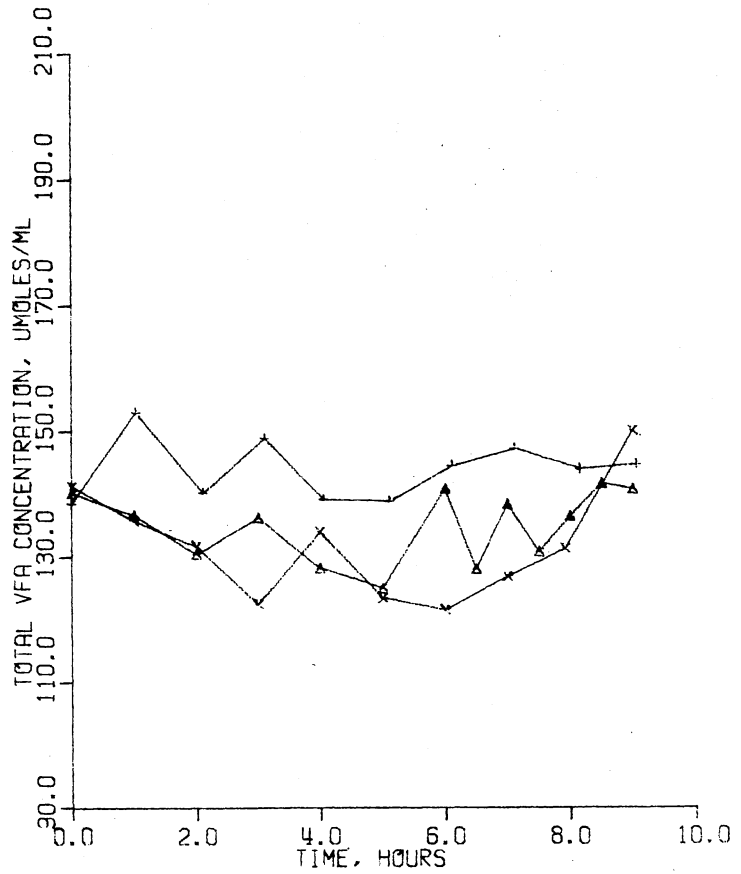
Figure 35. (Continued)

Figure 36. Ruminal Total Volatile Fatty Acid Concentration versus Time. Animal-ration periods are indicated by (a) 608-WC, (b) 422-WC, (c) 612-GC, (d) 608-GC.

(a) TOTAL VFA CONCENTRATION VS TIME

ANIMAL = 608
RATION = WC

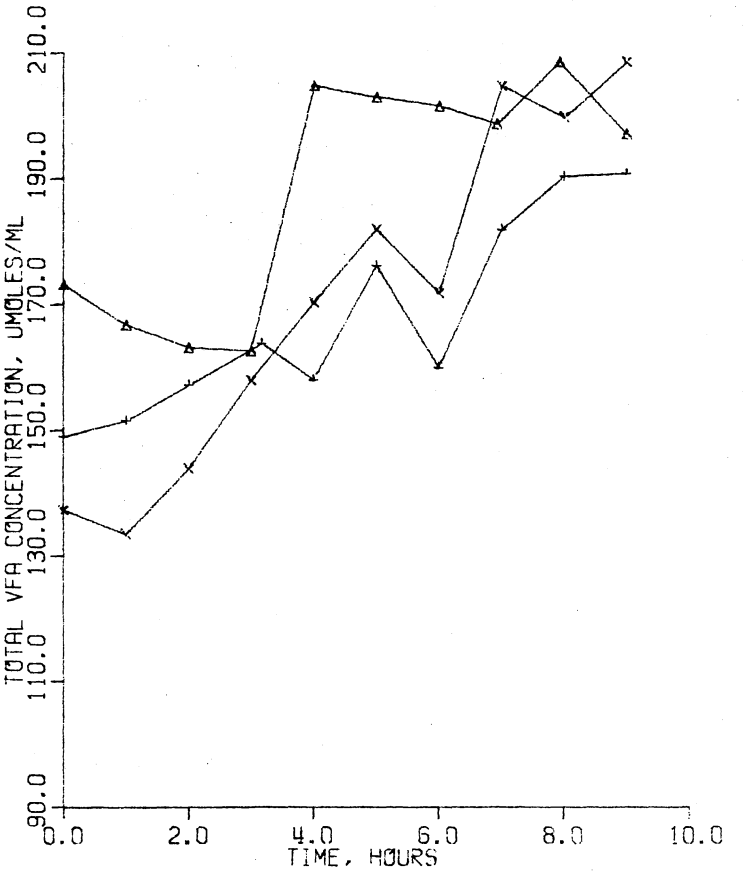
ACETATE EXPT = +
PROPIONATE EXPT = ×
BUTYRATE EXPT = △

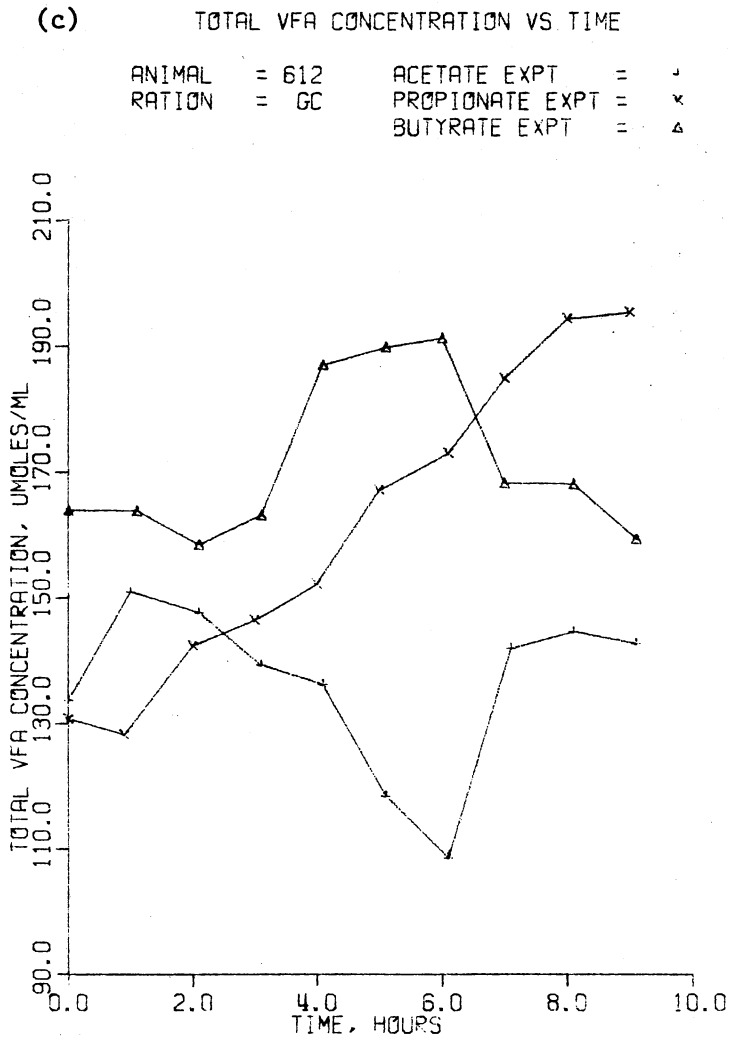


(b) TOTAL VFA CONCENTRATION VS TIME

ANIMAL = 422
RATION = WC

ACETATE EXPT = +
PROPIONATE EXPT = ×
BUTYRATE EXPT = △





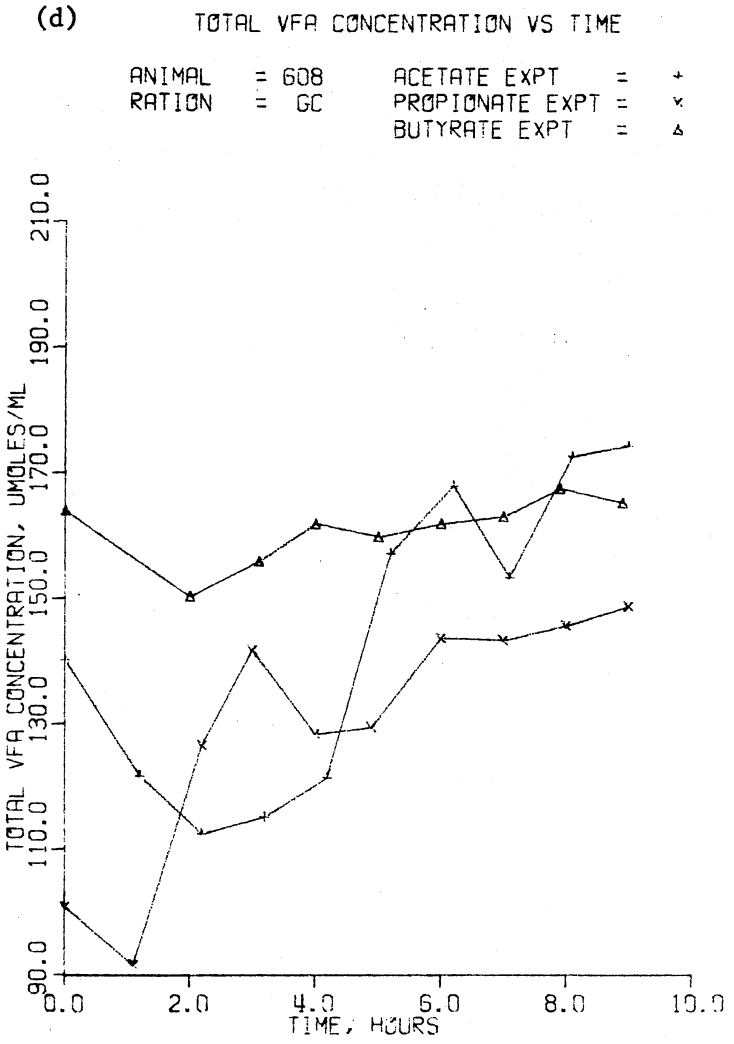


Figure 36. (Continued)

TABLE XXX

MEAN RUMINAL VFA CONCENTRATION DURING EACH INFUSION EXPERIMENT

Animal	Diet	Expt	n	VFA Concentration[a]								Ac/Pr
				Ac	Pr	iB	Bu	iV[b]	V	C	total	
608	WC	Ac	10	83.10 ±3.57	26.37 ±4.32	1.65 ±12.3	21.24 ±4.18	5.31 ±10.7	4.02 ±9.15	2.71 ±6.33	143.86 ±2.72	3.15
608	WC	Pr	10	75.56 ±7.35	26.34 ±9.26	2.21 ±8.98	19.15 ±7.57	5.40 ±5.50	2.25 ±12.3	0.85 ±25.6	131.76 ±6.98	2.87
608	WC	Bu	13	78.94 ±4.38	30.34 ±6.36	1.86 ±8.39	16.03 ±5.36	3.85 ±8.48	2.80 ±16.4	1.02 ±30.5	134.83 ±4.42	2.60
422	WC	Ac	10	55.76 ±19.8	77.07 ±8.32	1.03 ±13.7	26.56 ±7.72	1.56 ±13.9	4.34 ±12.0	1.64 ±9.35	167.95 ±10.5	0.72
422	WC	Pr	10	56.11 ±15.0	70.63 ±15.8	1.09 ±17.1	33.21 ±17.9	1.64 ±15.0	5.94 ±17.3	2.41 ±17.2	171.02 ±15.9	0.79
422	WC	Bu	10	63.52 ±9.86	73.83 ±11.2	1.01 ±6.86	39.41 ±9.16	2.24 ±5.93	5.55 ±11.7	2.43 ±7.55	187.98 ±9.94	0.86
612	GC	Ac	10	54.12 ±10.2	59.24 ±9.93	1.12 ±8.64	15.55 ±10.4	2.07 ±7.15	2.87 ±9.10	1.40 ±11.4	136.36 ±9.64	0.91
612	GC	Pr	10	70.12 ±13.0	72.24 ±18.1	1.02 ±15.4	12.56 ±21.8	1.49 ±10.9	2.90 ±20.2	1.13 ±21.2	161.45 ±15.1	0.97
612	GC	Bu	10	64.88 ±8.08	85.99 ±7.13	1.10 ±12.8	14.24 ±10.8	1.42 ±8.10	2.83 ±9.61	0.83 ±5.43	171.29 ±7.54	0.76
608	GC	Ac	10	51.94 ±18.5	71.06 ±17.0	1.00 ±14.3	14.84 ±18.9	1.98 ±8.98	2.20 ±13.7	0.65 ±3.87	143.67 ±15.7	0.73
608	GC	Pr	10	51.06 ±15.4	60.98 ±16.0	0.96 ±11.3	11.72 ±8.50	1.61 ±8.56	2.65 ±9.95	1.00 ±7.23	129.98 ±14.6	0.84
608	GC	Bu	9	90.51 ±4.60	27.56 ±8.18	0.99 ±3.94	34.07 ±10.5	4.97 ±6.30	1.94 ±11.3	1.05 ±15.5	161.08 ±3.67	3.28

[a] Values are expressed in μmoles/ml; (infusion mean ± % coefficient of variation).

[b] Contains 2-methylbutyric and 3-methylbutyric acids.

TABLE XXXI

MEAN VFA CONCENTRATION FOR EACH ANIMAL-RATION PERIOD

Period	n	VFA Concentration (μmoles/ml)								Ac/Pr
		Ac	Pr	iB	Bu	iV	V	c	total	
608-WC	3	79.20[b]	27.68[b]	1.904[b]	18.81[b]	4.853[b]	3.024[b]	1.344[b]	136.82[b]	2.86[b]
422-WC	3	58.46[c]	73.84[c]	1.039[c]	33.06[c]	1.814[c]	5.273[c]	2.156[b]	175.65[c]	0.79[c]
612-GC	3	63.04[b]	72.49[b]	1.081[b]	14.12[b]	1.660[b]	2.866[b]	1.120[b]	156.37[b]	0.87[b]
608-GC	3	64.50[b]	58.20[b]	0.982[c]	20.21[b]	2.854[b]	2.265[c]	0.897[b]	144.91[b]	1.21[b]
608-GC[a]	2	51.50[b]	66.02[b]	0.977[b]	13.28[b]	1.798[b]	2.248[b]	0.821[b]	136.83[b]	0.78[b]
WC	6	68.83[d]	50.76[d]	1.472[d]	25.93[d]	3.333[d]	4.148[d]	1.750[d]	156.23[d]	1.36[d]
GC	6	63.77[d]	62.85[d]	1.031[d]	17.16[d]	2.257[d]	2.566[e]	1.008[e]	150.64[d]	1.01[d]
GC[a]	5	57.27[d]	69.26[d]	1.029[d]	13.70[d]	1.729[d]	2.647[d]	0.971[d]	146.60[d]	0.83[d]
WC SE[f]	6	5.57	9.29	0.15	3.55	0.64	0.44	0.21	8.07	
GC SE[g]	6	4.32	8.26	0.16	2.76	0.54	0.45	0.25	8.36	
GC SE[g]	5	4.73	9.05	0.17	3.02	0.60	0.49	0.28	9.16	

[a]The 608-Bu-GC infusion was deleted from calculations.

[b,c]Comparisons between animals within a ration type with different superscripts are different ($P < .05$).

[d,e]Comparisons between whole and ground corn with different superscripts are different ($P < .05$).

[f]Standard error of the mean.

[g]Standard error of the mean (calculations not including 608-Bu-GC data).

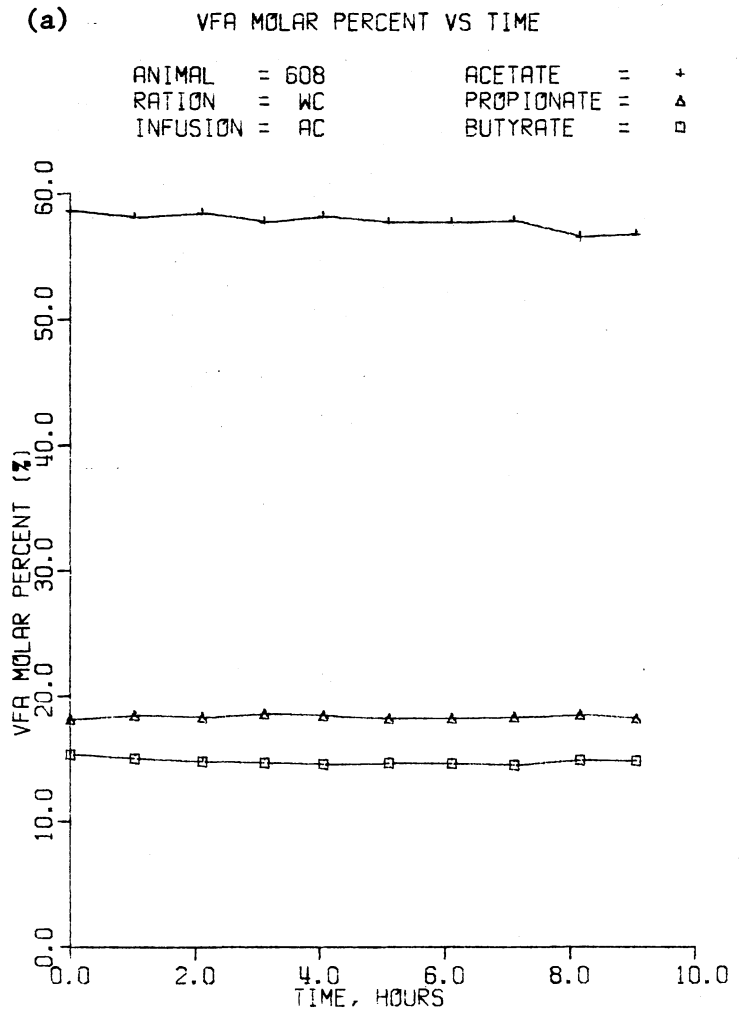
total VFA concentration than did Animal 608, suggesting the presence of a more intense fermentation rate.

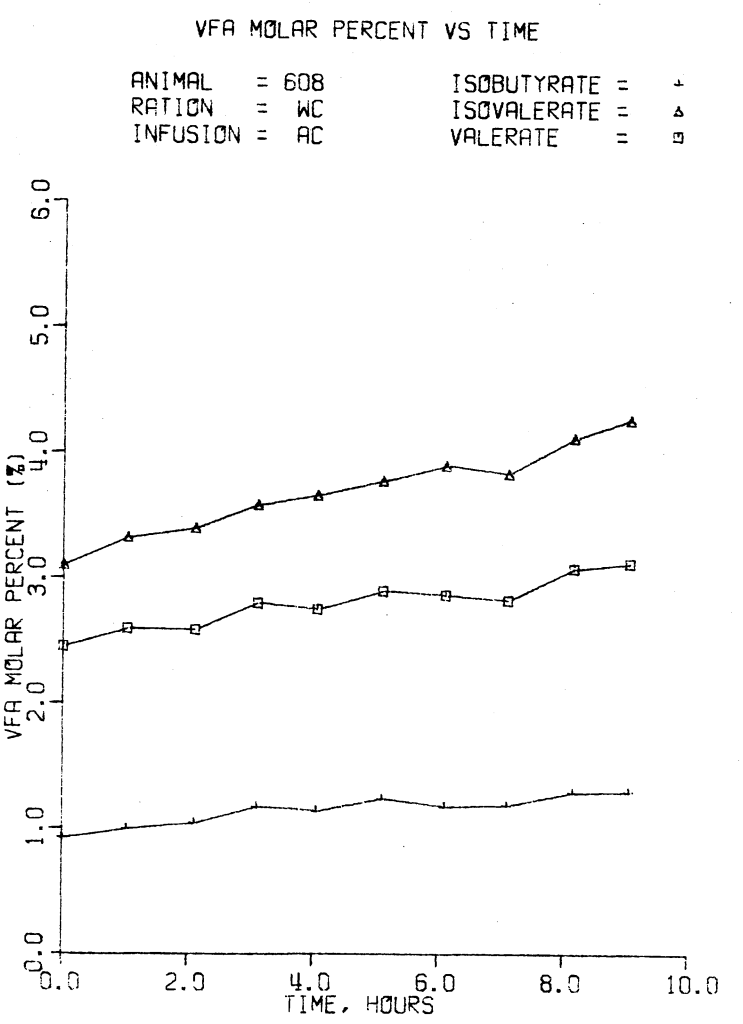
VFA patterns were more uniform in steers consuming the GC ration. It should be pointed out that the fermentation pattern of Animal 608 during the butyrate infusion was markedly different than that observed during the other infusions with this animal and Animal 612. Table XXX indicates that during the 608-GC-Bu infusion a high Ac/Pr (3.28) was observed coupled with an elevated (approx. 2.5X) butyric acid concentration as compared with that on the other GC infusions. Accompanying this apparently altered fermentation was a noticeable change in the physical properties of the rumen fluid; i.e., the consistency of the fluid was very viscous and stringy suggesting the presence of slime forming bacteria. Consequently, sampling was impeded. If the 608-GC-Bu infusion is removed from the comparison, VFA patterns between the two animals on the ground corn diet were very similar (Table XXXI).

Some of the variation in VFA concentration can probably be attributed to variation in the site of sampling (Sutton, 1972) since true steady-state conditions are rarely achieved due to the heterogeneity (i.e., stratification) of the rumen contents. Frequent feeding of high-concentrate diets may result in the presence of a pseudo-steady-state condition in which fermentation in localized areas of the rumen may be considered to exist in steady-state, but the rate and/or extent of fermentation may vary with site. Respective comparisons of the VFA concentration over time, shown in Figures 32-35, or the VFA molar percent over time, illustrated in Figures 37-40, seem to support this hypothesis. This is further substantiated by the lower coefficients of variation for percentage concentrations shown in Table XXXII as

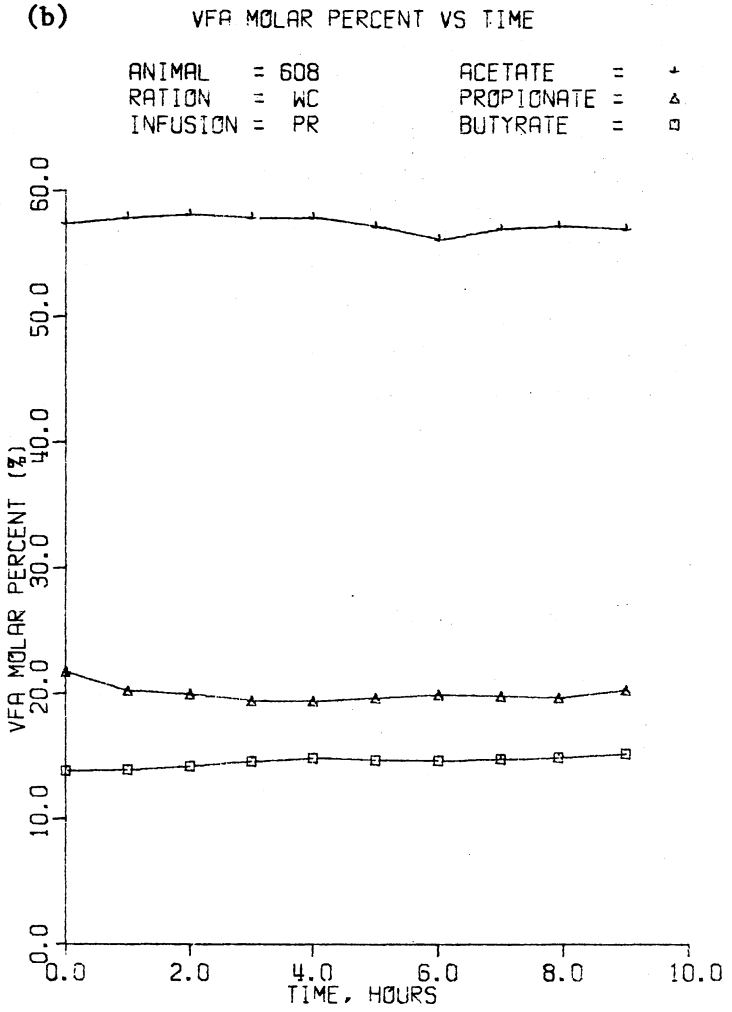
Figure 37. Ruminal VFA Molar Percent versus Time for Animal 608 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)


VFA MOLAR PERCENT VS TIME
ANIMAL = 608
RATION = WC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA MOLAR PERCENT (%)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


VFA MOLAR PERCENT VS TIME
ANIMAL = 608
RATION = WC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA MOLAR PERCENT (%)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



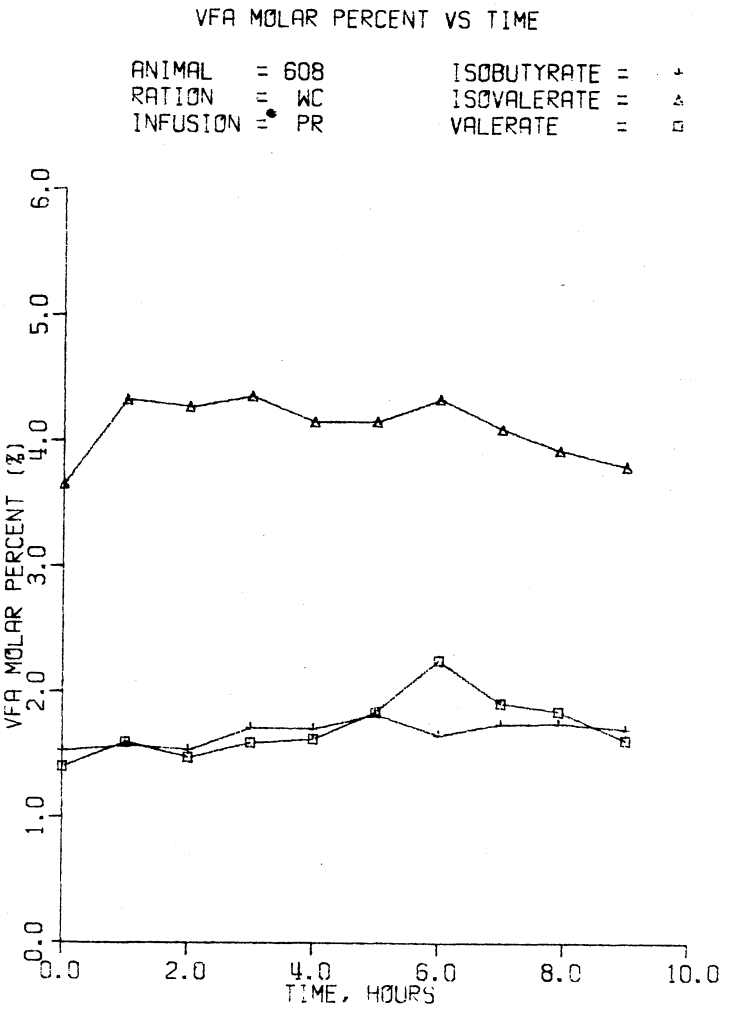
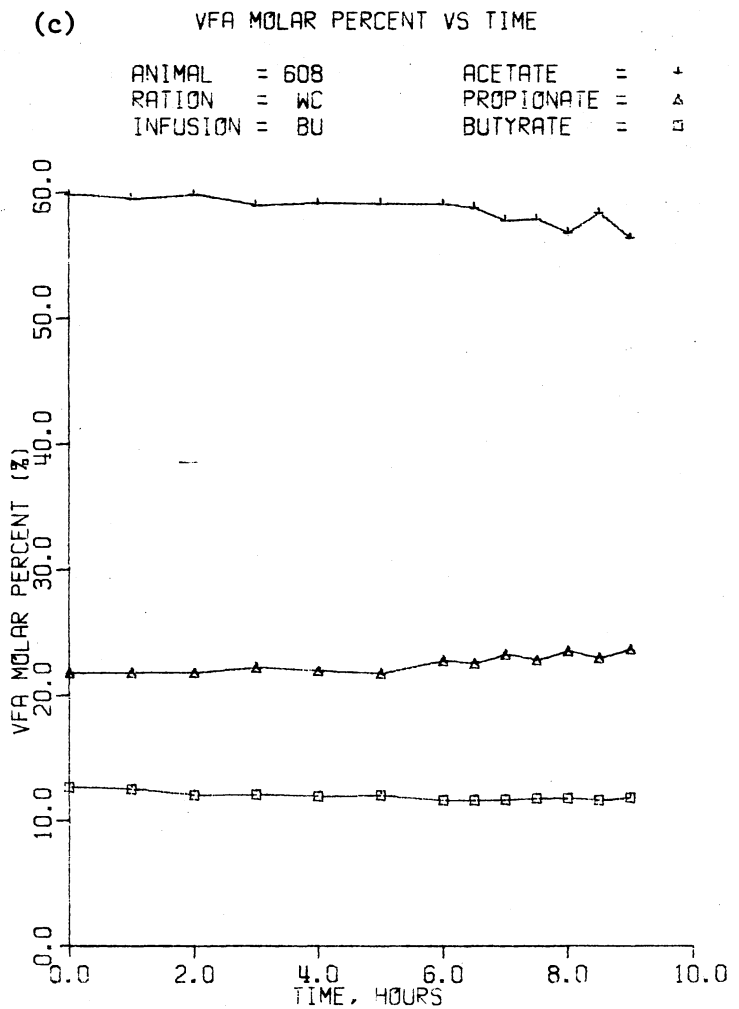


Figure 37. (Continued)

(c)



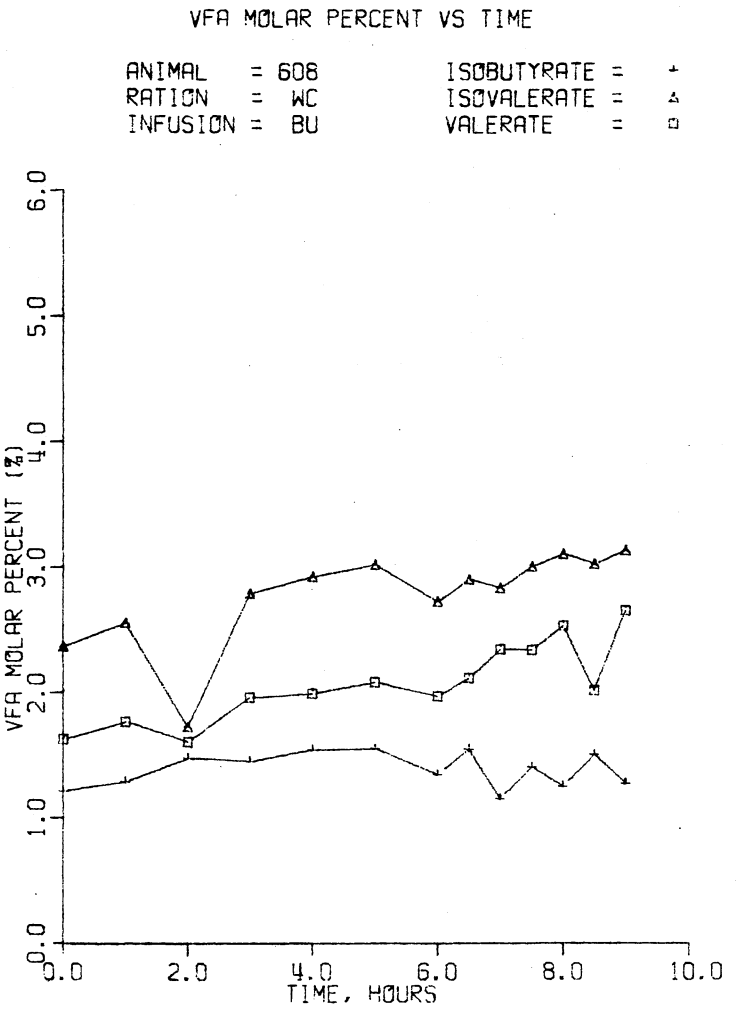
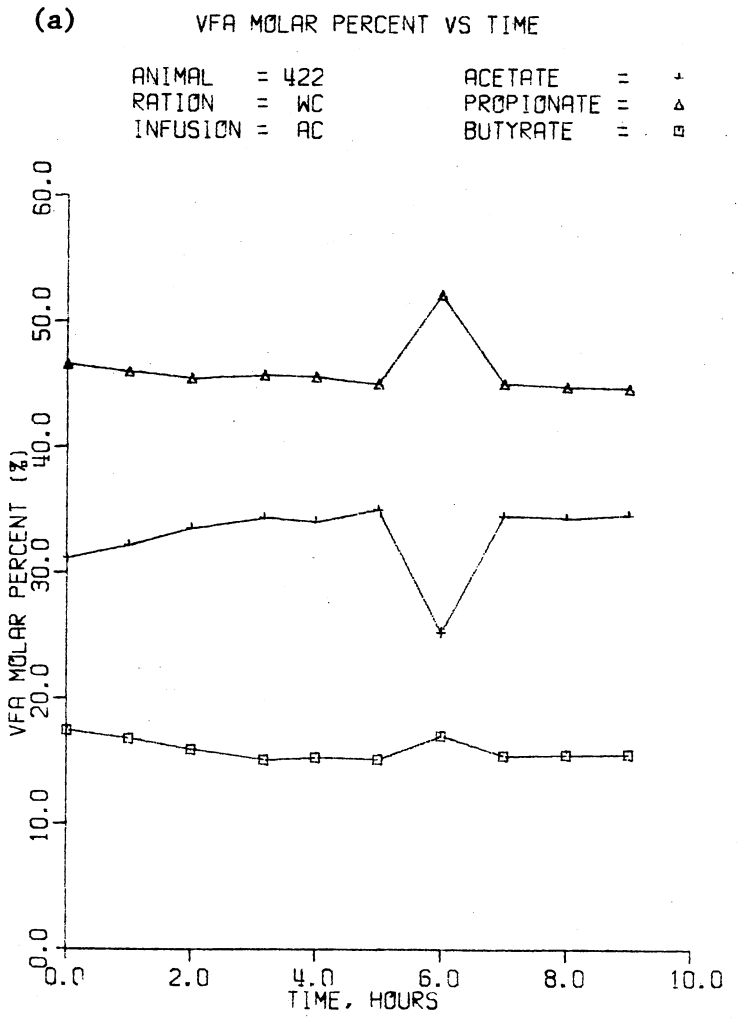
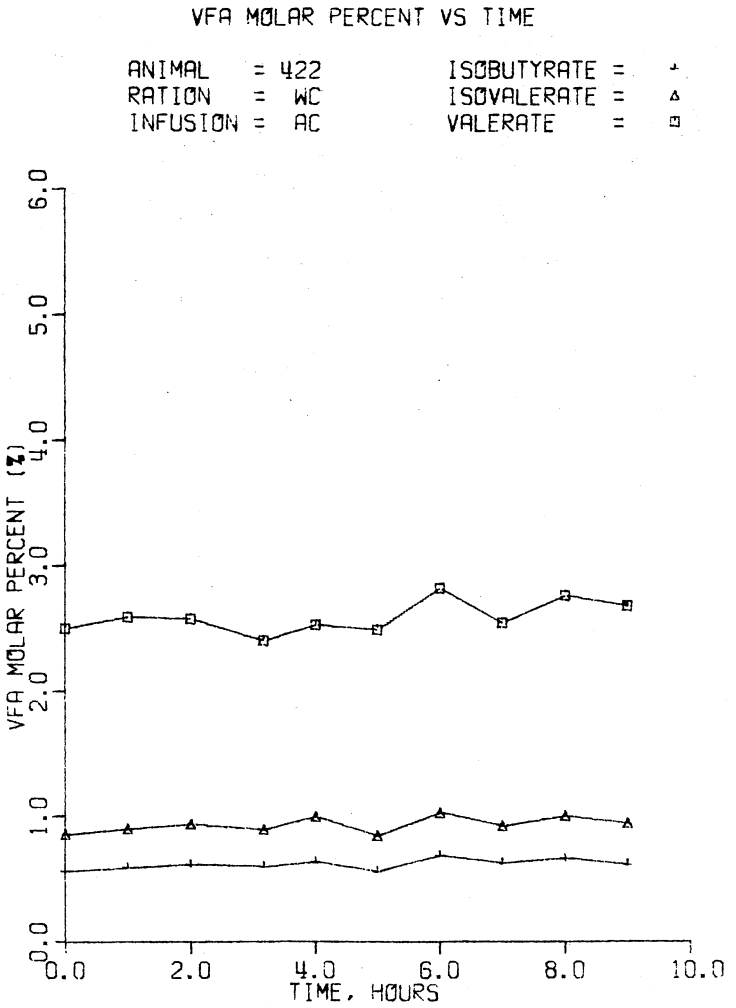


Figure 37. (Continued)

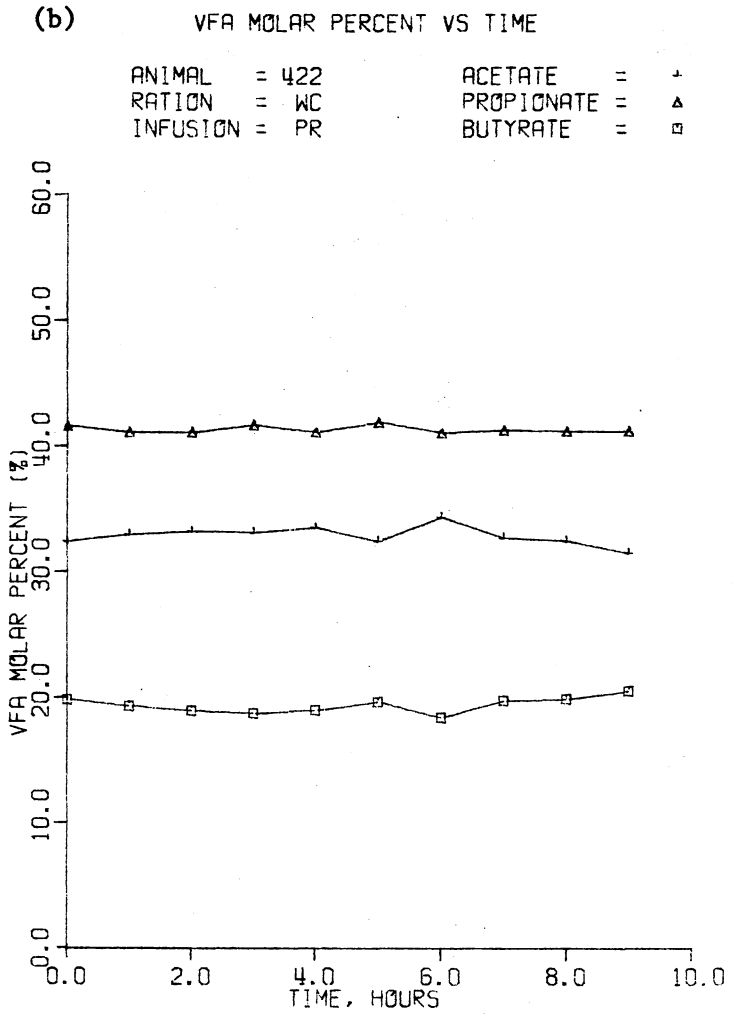
Figure 38. Ruminal VFA Molar Percent versus Time for Animal 422 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)


VFA MOLAR PERCENT VS TIME
ANIMAL = 422
RATION = WC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA MOLAR PERCENT (%)
0.0
10.0
20.0
30.0
40.0
50.0
60.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


VFA MOLAR PERCENT VS TIME
ANIMAL = 422
RATION = WC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA MOLAR PERCENT (%)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



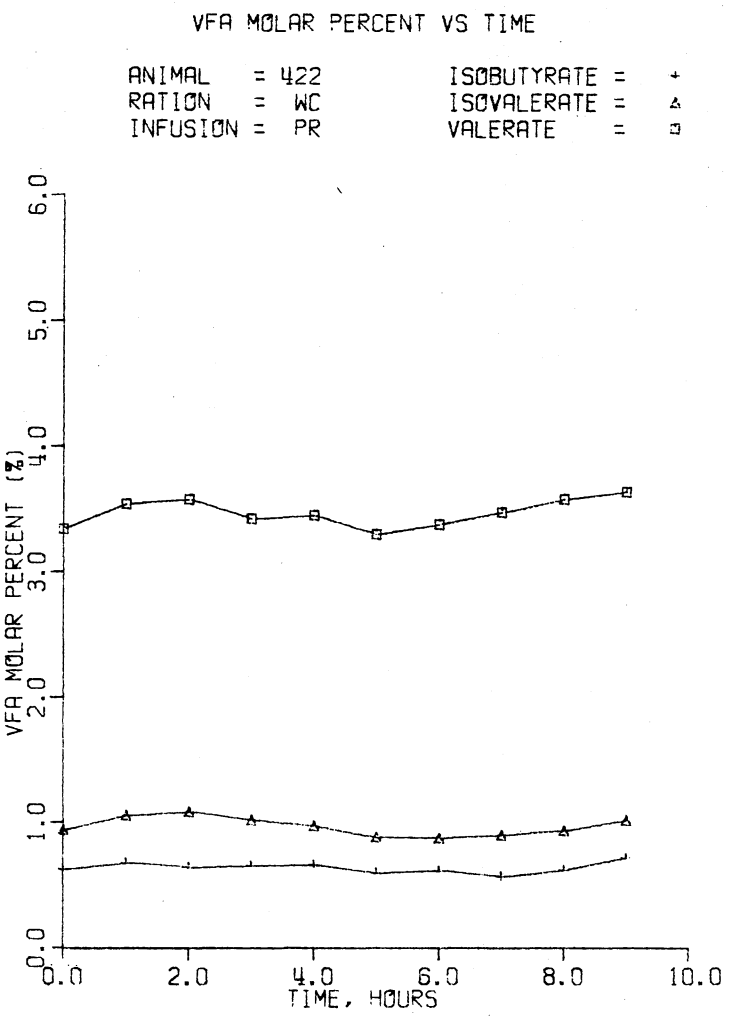
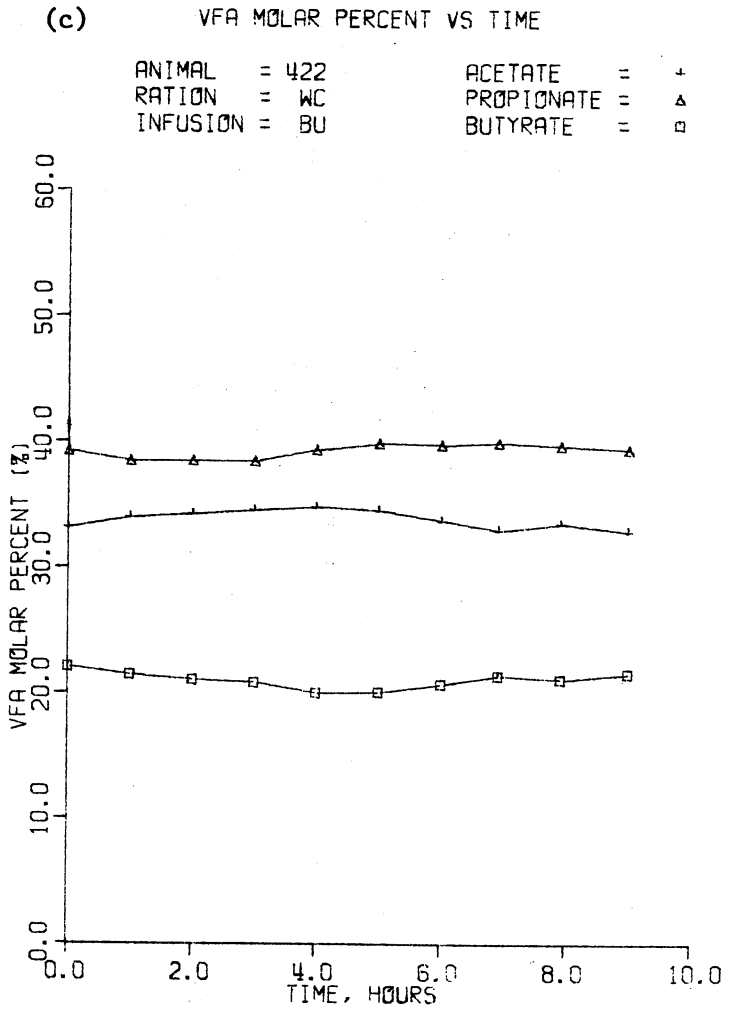


Figure 38. (Continued)

(c)



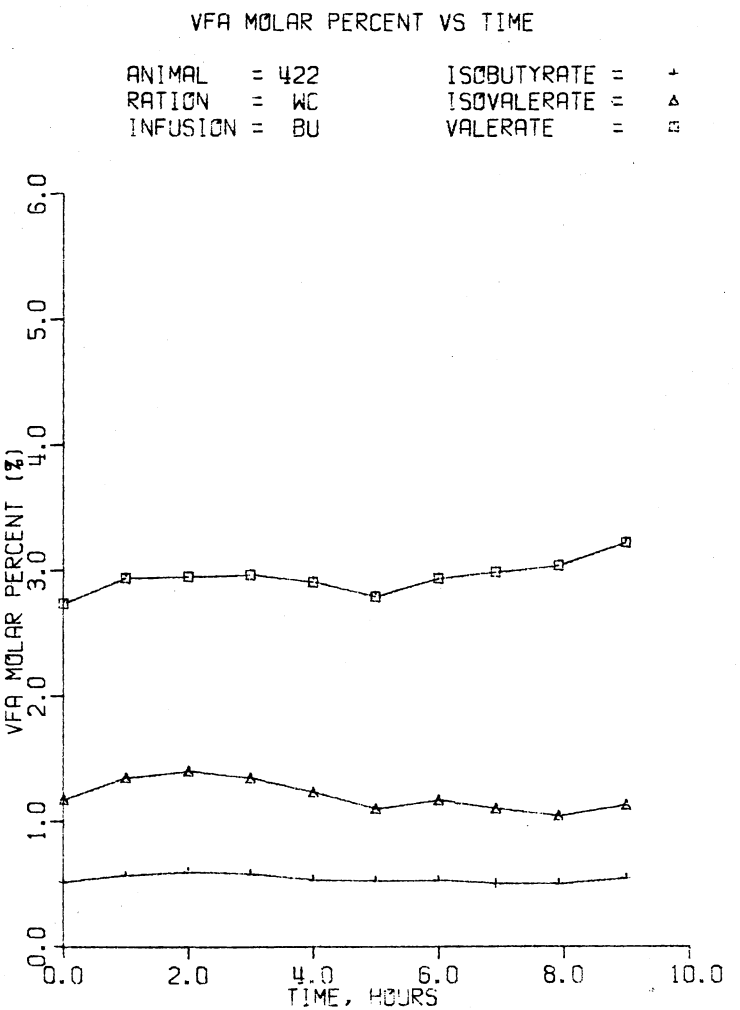
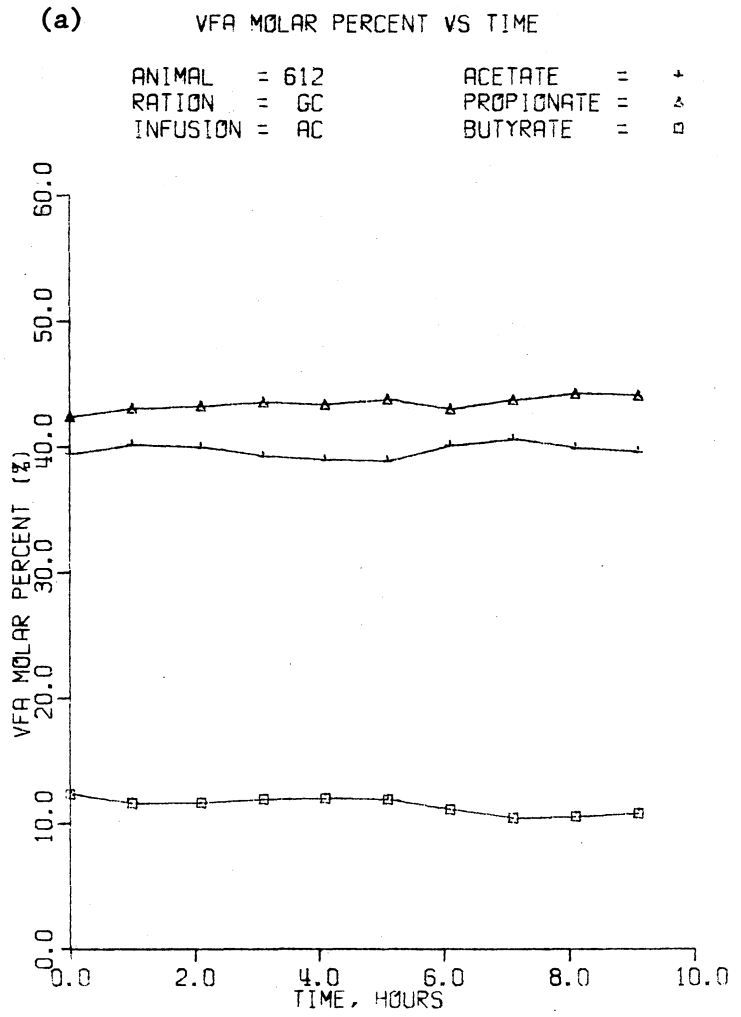
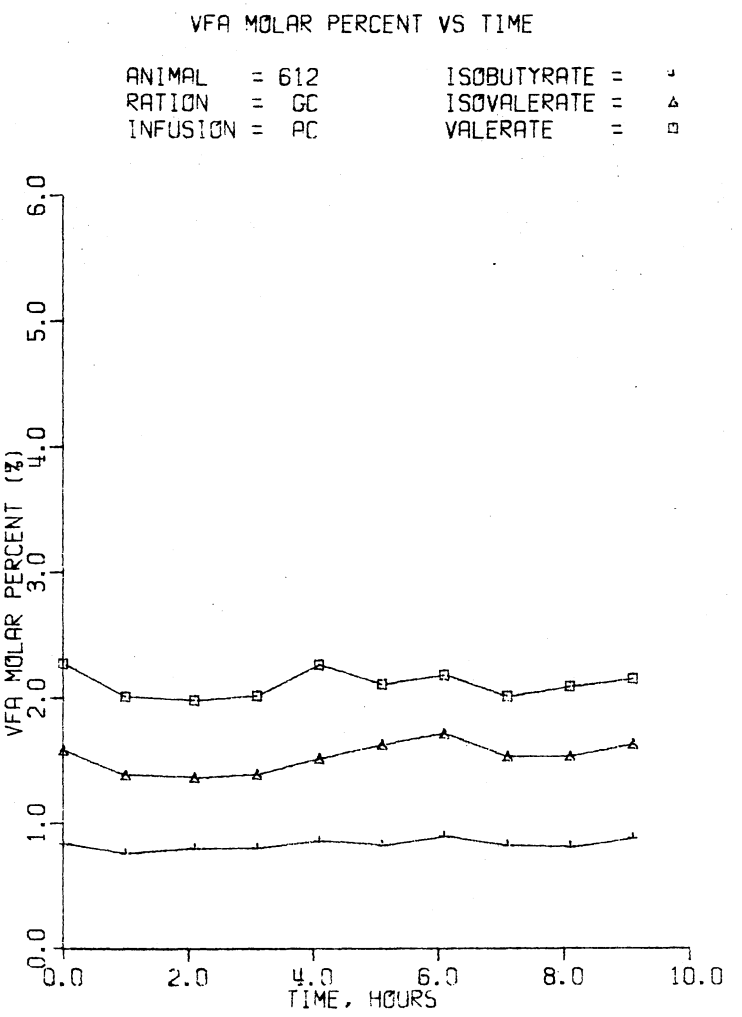


Figure 38. (Continued)

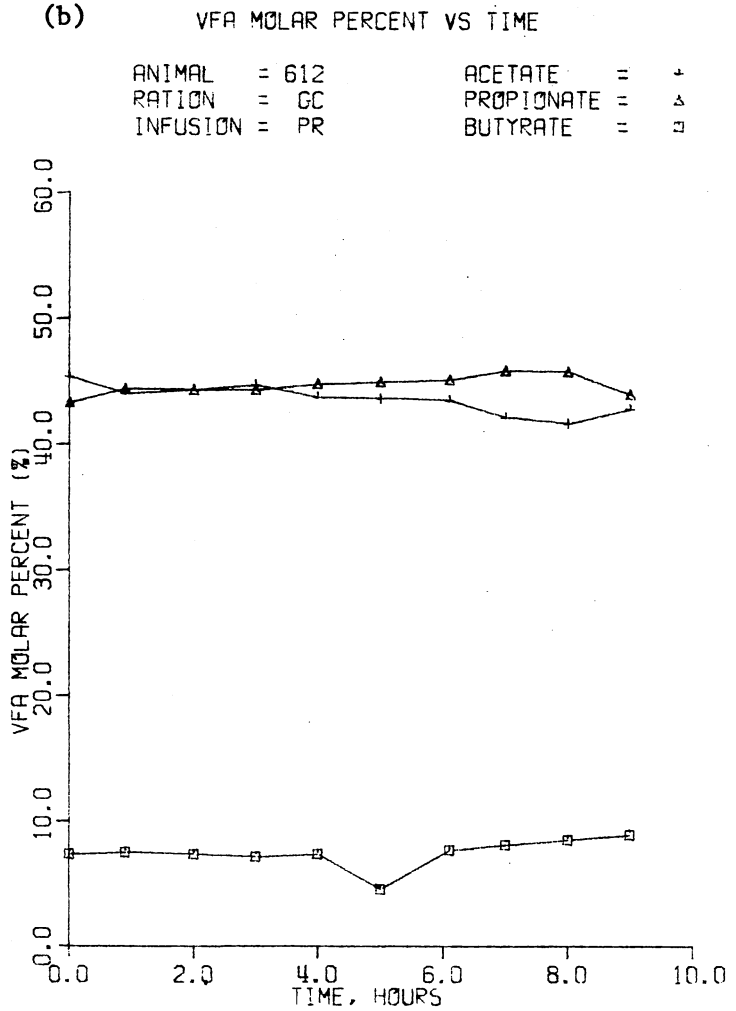
Figure 39. Ruminal VFA Molar Percent versus Time for Animal 612 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)


VFA MOLAR PERCENT VS TIME
ANIMAL = 612
RATION = GC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA MOLAR PERCENT (%)
0.0
10.0
20.0
30.0
40.0
50.0
60.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


VFA MOLAR PERCENT VS TIME
ANIMAL = 612
RATION = GC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA MOLAR PERCENT (%)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



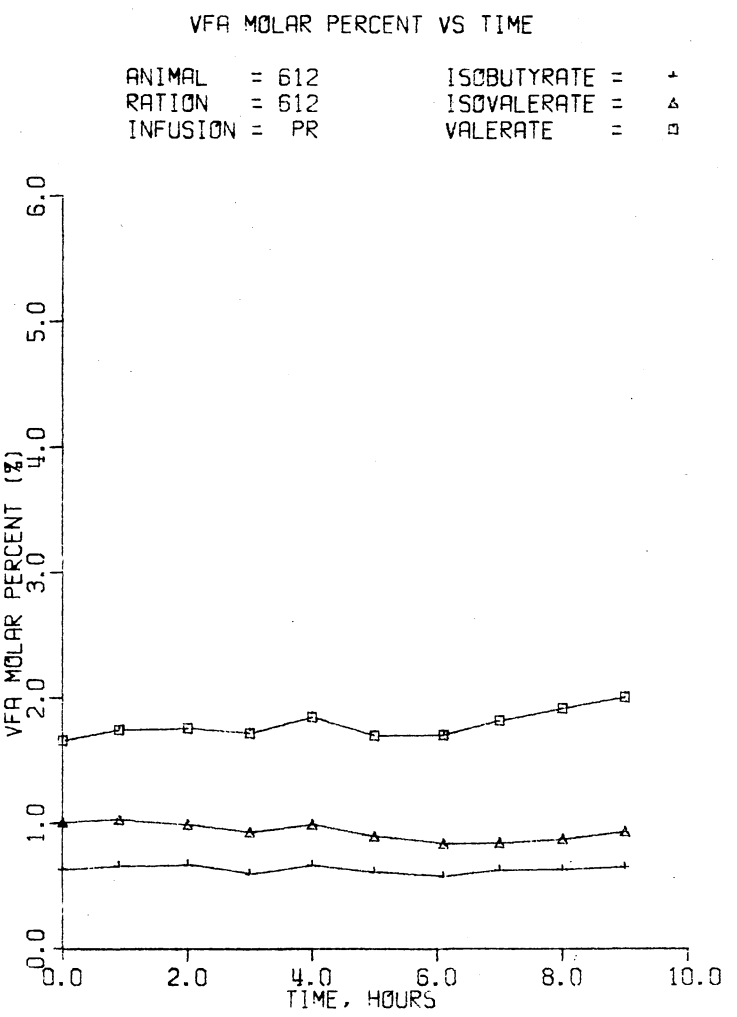
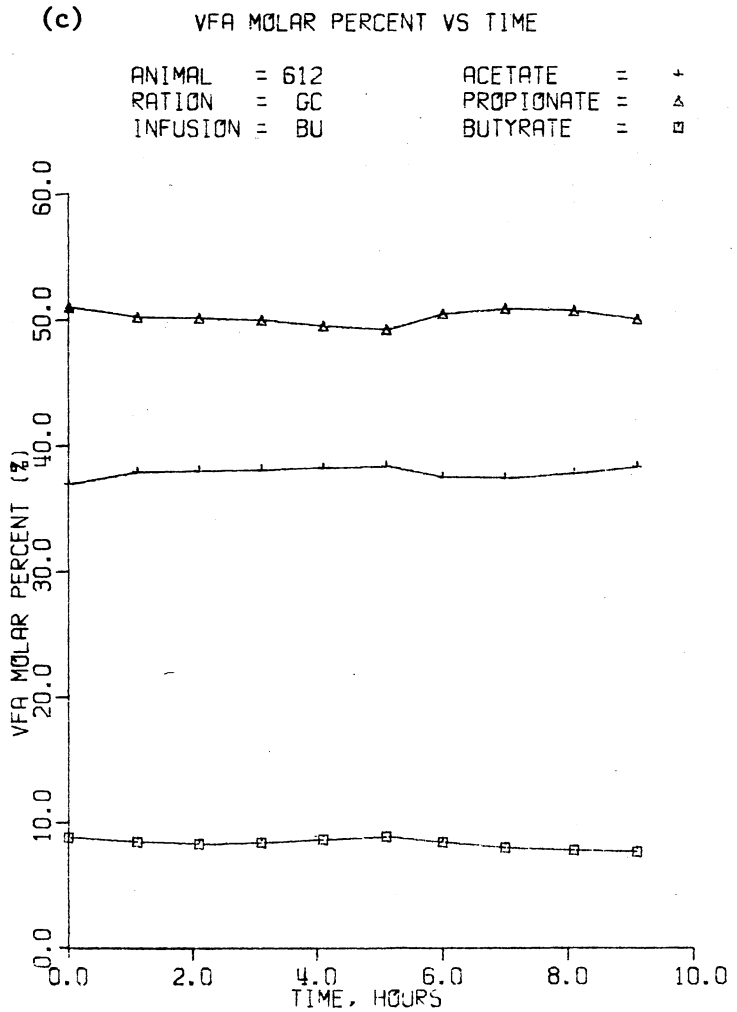


Figure 39. (Continued)

(c)



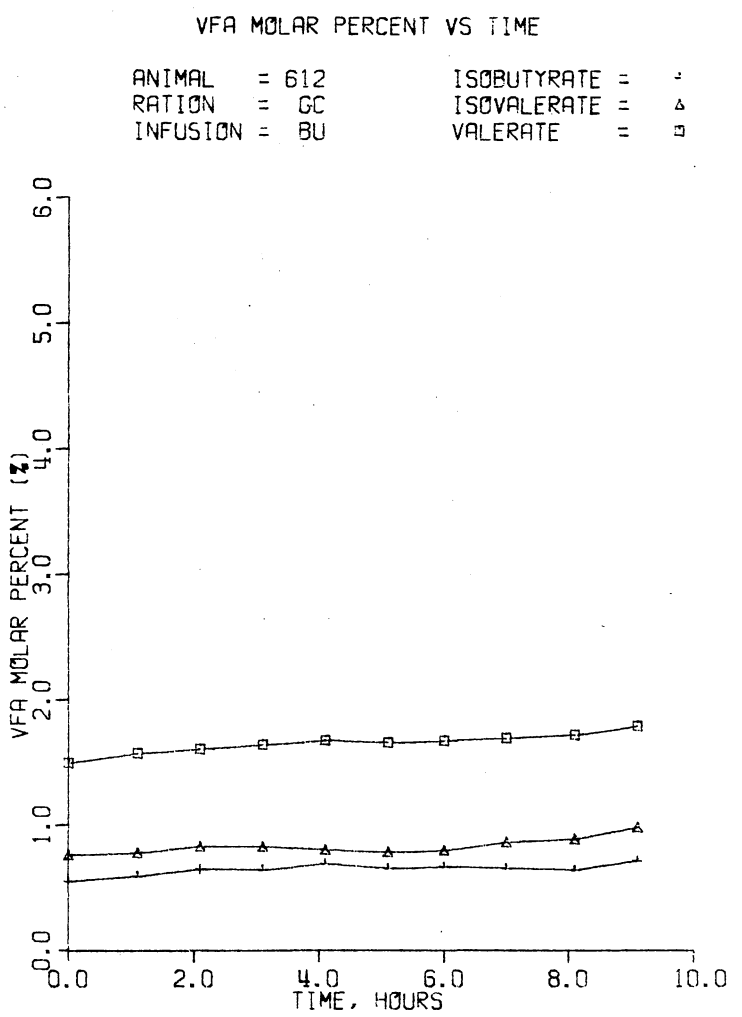
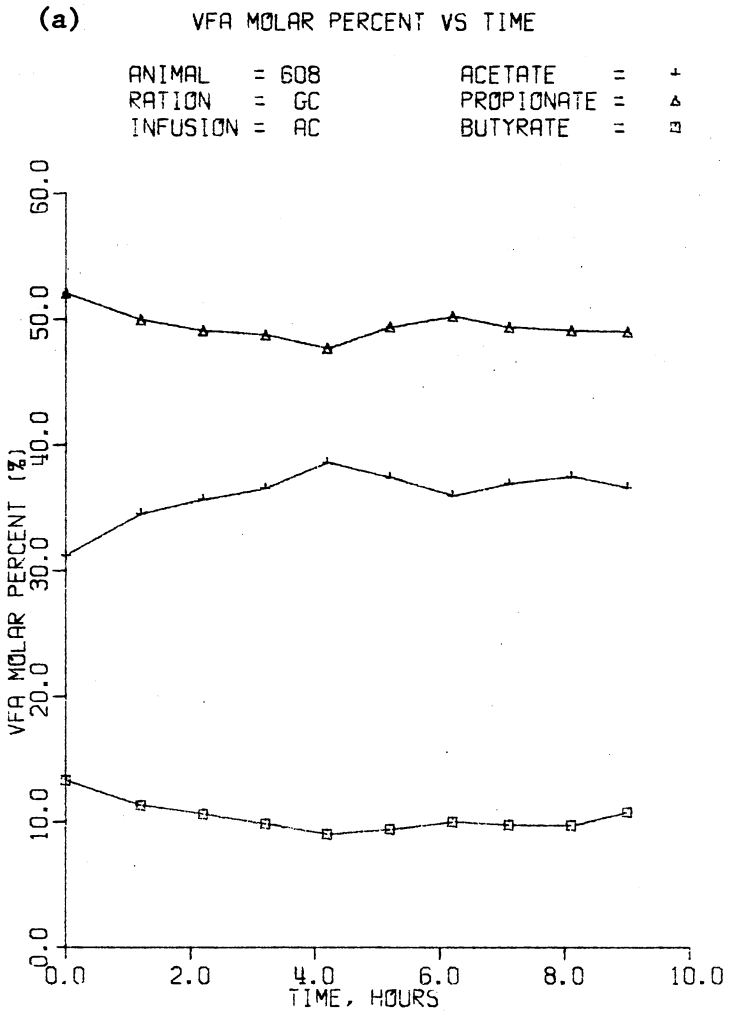
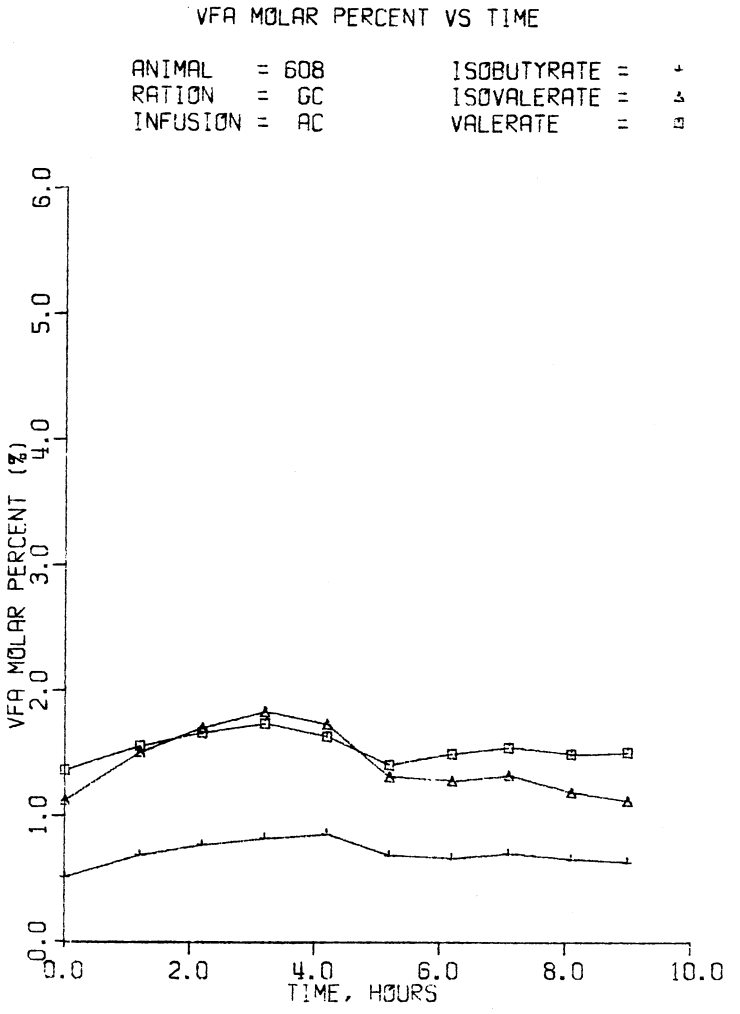


Figure 39. (Continued)

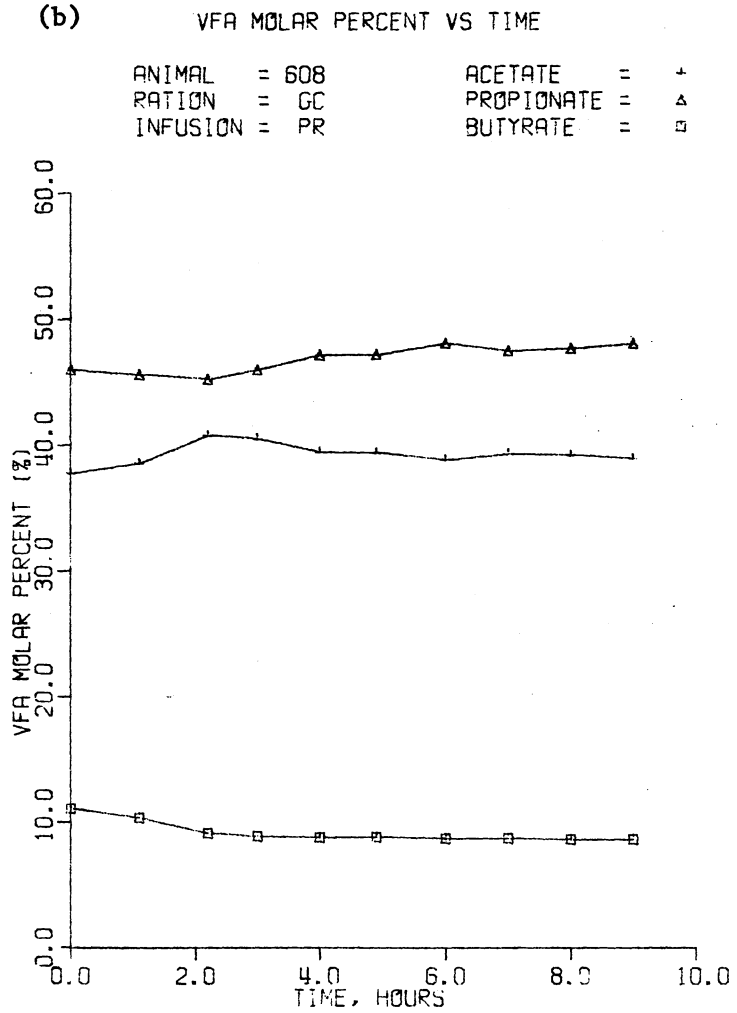
Figure 40. Ruminal VFA Molar Percent versus Time for Animal 608 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment.

(a)


VFA MOLAR PERCENT VS TIME
ANIMAL = 608
RATION = GC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
VFA MOLAR PERCENT (%)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


VFA MOLAR PERCENT VS TIME
ANIMAL = 608
RATION = GC
INFUSION = AC
ISOBUTYRATE =
ISOVALERATE =
VALERATE =
VFA MOLAR PERCENT (%)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)



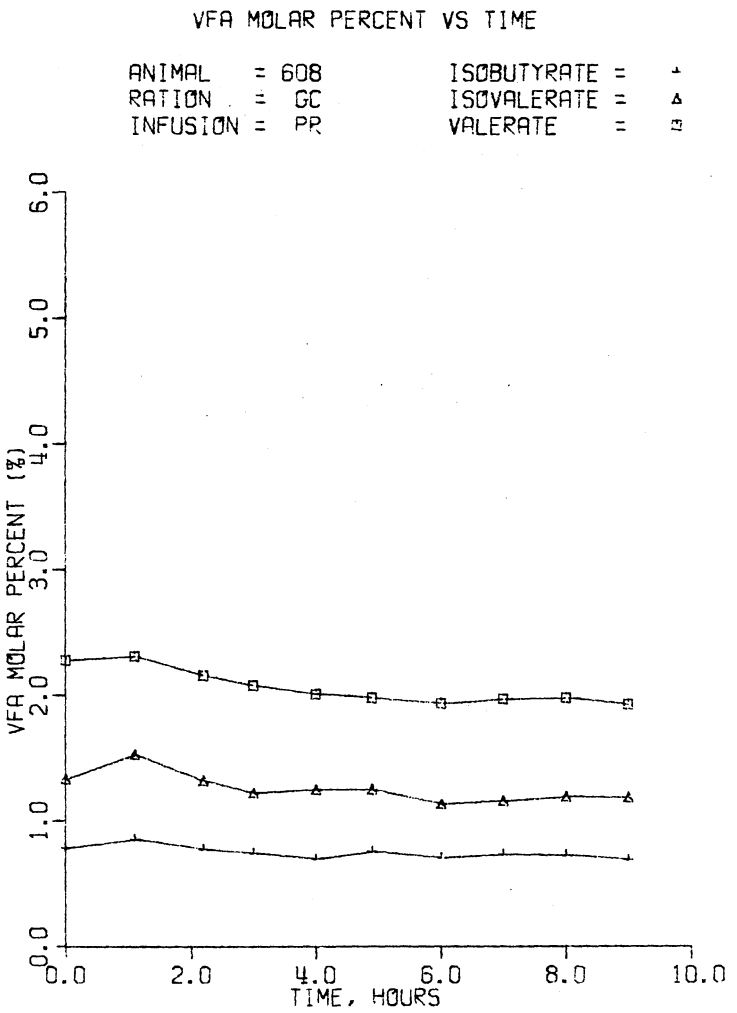
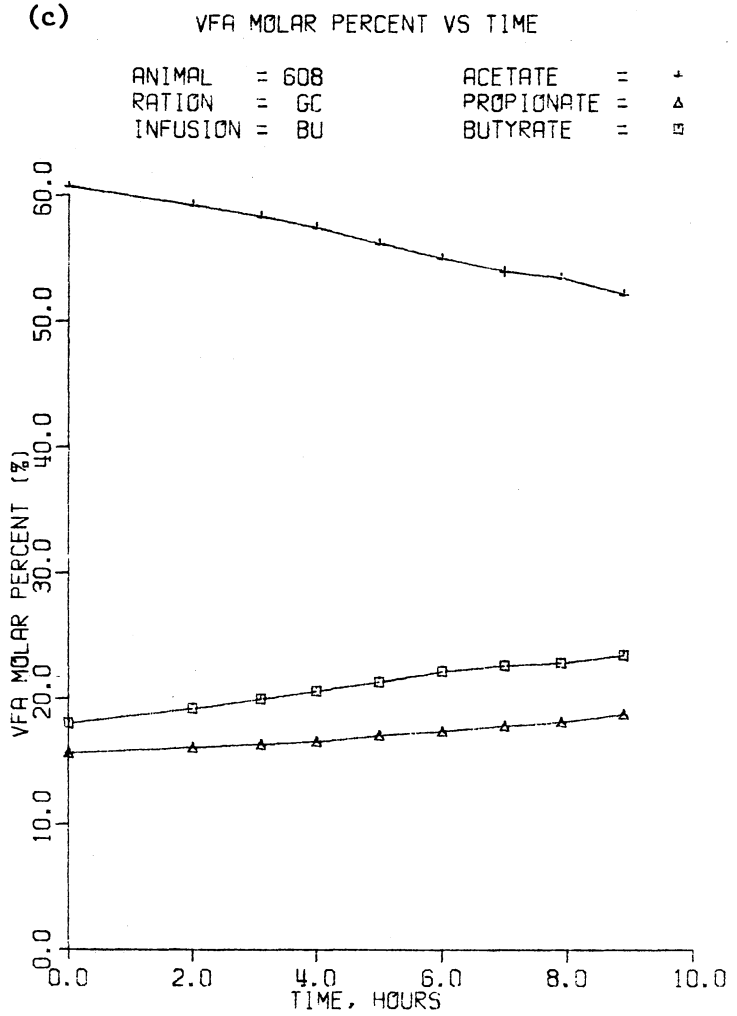


Figure 40. (Continued)

(c)



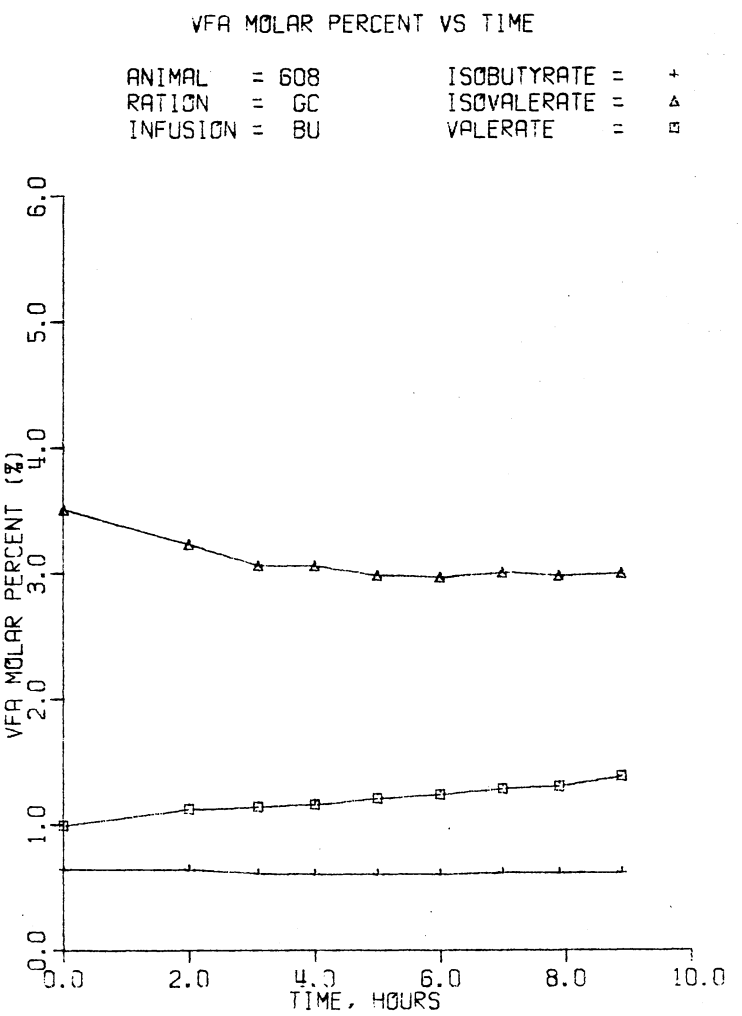


Figure 40. (Continued)

TABLE XXXII

MEAN RUMINAL VFA MOLAR PERCENT DURING EACH INFUSION EXPERIMENT

Animal	Diet	Expt	n	Molar Percent[a]						
				Ac	Pr	iB	Bu	iV[b]	V	C
608	WC	Ac	10	57.77 ±1.24	18.33 ±1.10	1.14 ±11.4	14.76 ±1.80	3.69 ±9.67	2.79 ±7.78	1.50 ±4.27
608	WC	Pr	10	57.34 ±1.24	19.97 ±3.31	1.68 ±8.51	14.53 ±2.97	4.11 ±6.50	1.72 ±15.3	0.65 ±30.3
608	WC	Bu	13	58.56 ±1.84	22.49 ±3.01	1.38 ±10.2	11.89 ±2.89	2.85 ±8.00	2.08 ±15.2	0.75 ±28.9
422	WC	Ac	10	32.91 ±14.8	46.09 ±7.53	0.61 ±10.8	15.90 ±7.67	0.93 ±11.3	2.59 ±8.73	0.98 ±7.76
422	WC	Pr	10	32.85 ±2.33	41.30 ±0.80	0.64 ±7.19	19.37 ±8.20	0.96 ±8.23	3.46 ±3.44	1.41 ±5.53
422	WC	Bu	10	33.80 ±2.01	39.22 ±1.81	0.54 ±6.11	20.99 ±2.99	1.20 ±10.0	2.95 ±4.41	1.29 ±5.35
612	GC	Ac	10	39.67 ±1.45	42.43 ±1.30	0.83 ±5.89	11.41 ±5.77	1.53 ±7.65	2.10 ±5.71	1.03 ±6.80
612	GC	Pr	10	43.57 ±2.67	44.68 ±1.87	0.63 ±6.46	7.71 ±7.12	0.93 ±7.53	1.79 ±6.48	0.69 ±6.96
612	GC	Bu	10	37.86 ±1.87	50.22 ±1.73	0.64 ±9.63	8.30 ±5.00	0.83 ±8.55	1.65 ±5.50	0.48 ±3.54
608	GC	Ac	10	36.09 ±5.50	49.43 ±2.28	0.70 ±13.7	10.35 ±11.6	1.42 ±18.0	1.54 ±7.27	0.46 ±14.8
608	GC	Pr	10	39.23 ±8.25	48.82 ±2.20	0.74 ±7.03	9.12 ±9.00	1.26 ±8.81	2.06 ±6.70	0.78 ±9.74
608	GC	Bu	9	56.23 ±4.93	17.09 ±5.71	0.62 ±2.58	21.12 ±8.33	3.08 ±5.58	1.20 ±9.42	0.650 ±13.7

[a]Values are the mean of each experiment ± % coefficient of variation.

[b]Contains 2-methylbutyric and 3-methylbutyric acids.

compared to absolute concentrations in Table XXX.

Table XXXIII summarizes the VFA molar percent data across each animal-ration period and ration type. Again, it is clear that Animal 422 had a different type of rumen fermentation as compared to Animal 608 when both were fed the WC diet. Generally, these differences were significant ($P < .05$). In contrast, the animals consuming the GC ration exhibited a more consistent VFA pattern. When WC was compared to GC, there tended to be higher proportions of acetate, butyrate and branched and straight chain ($C > 4$) VFA's and a lower proportion of propionate on the WC diet. This is in agreement with Vance et al. (1970) and White et al. (1972). Values for Animal 608 (WC) are similar to those reported by Cole et al. (1976a) for steers consuming similar WC rations. On the other hand, the observations for Animal 422 and Animals 612 and 608 (GC) are consistent with those reported by Rounds (1973) for steers consuming similar whole and ground corn rations, respectively. The reasons for such discrepancies are unclear.

Hodgson and Thomas (1975) suggested that a negative relationship existed between rumen dilution rate and the molar percentage of propionic acid in the rumen. The data illustrated in Figure 41 clearly does not support this hypothesis. Although rumen dilution rate may influence the numbers, types and metabolism of the bacterial population, it did not appear to be a primary determinant under the conditions used in this study.

Table XXXIV lists the ratio of 2-methylbutyric (2MB) to 3-methylbutyric (3MB) acid observed during each isotope infusion experiment. The importance of the branched chain VFA's and their relationship to amino acid catabolism and synthesis in the rumen ecosystem has been

TABLE XXXIII

MEAN VFA MOLAR PERCENT FOR EACH ANIMAL-RATION PERIOD

Period	n	Molar Percent						
		Ac	Pr	iB	Bu	iV	V	C
608-WC	3	57.89[b]	20.26[b]	1.40[b]	13.73[b]	3.55[b]	2.20[b]	0.97[b]
422-WC	3	33.19[c]	42.20[c]	0.60[c]	18.75[c]	1.03[c]	3.00[b]	1.23[b]
612-GC	3	40.37[b]	46.11[b]	0.70[b]	9.14[b]	1.10[b]	1.85[b]	0.73[b]
608-GC	3	43.85[b]	37.78[b]	0.69[b]	13.53[b]	1.92[b]	1.60[b]	0.63[b]
608-GC[a]	2	37.66[b]	48.13[b]	0.72[b]	10.96[b]	1.34[b]	1.80[b]	0.62[b]
WC	6	45.54[d]	31.23[d]	1.00[d]	16.24[d]	2.29[d]	2.60[d]	1.10[d]
GC	6	42.11[d]	41.95[d]	0.69[d]	11.34[d]	1.51[d]	1.72[e]	0.69[e]
GC[a]	5	39.28[d]	46.92[e]	0.71[d]	9.38 [e]	1.19[d]	1.83[e]	0.69[e]
WC SE[f]	6	4.45	5.05	0.14	1.73	0.48	0.20	0.12
GC SE[g]	6	4.19	3.83	0.15	1.10	0.45	0.20	0.13
GC SE[g]	5	4.59	4.19	0.16	1.21	0.49	0.22	0.14

[a]The 608-GC-Bu infusion data were excluded from the calculations.

[b,c]Comparisons between animals within a ration type with a different superscript are different ($P < .05$).

[d,e]Comparisons between whole and ground corn with different superscripts are different ($P < .05$).

[f]Standard error of the mean.

[g]Standard error of the mean (calculations not including 608-GC-Bu data).

Figure 41. The Relationship Between the Molar Proportion of Propionic Acid in the Rumen Fluid and the Dilution Rate of the Rumen Liquid Phase.

PROPIONIC ACID MOLAR % VS RUMEN DILUTION RATE

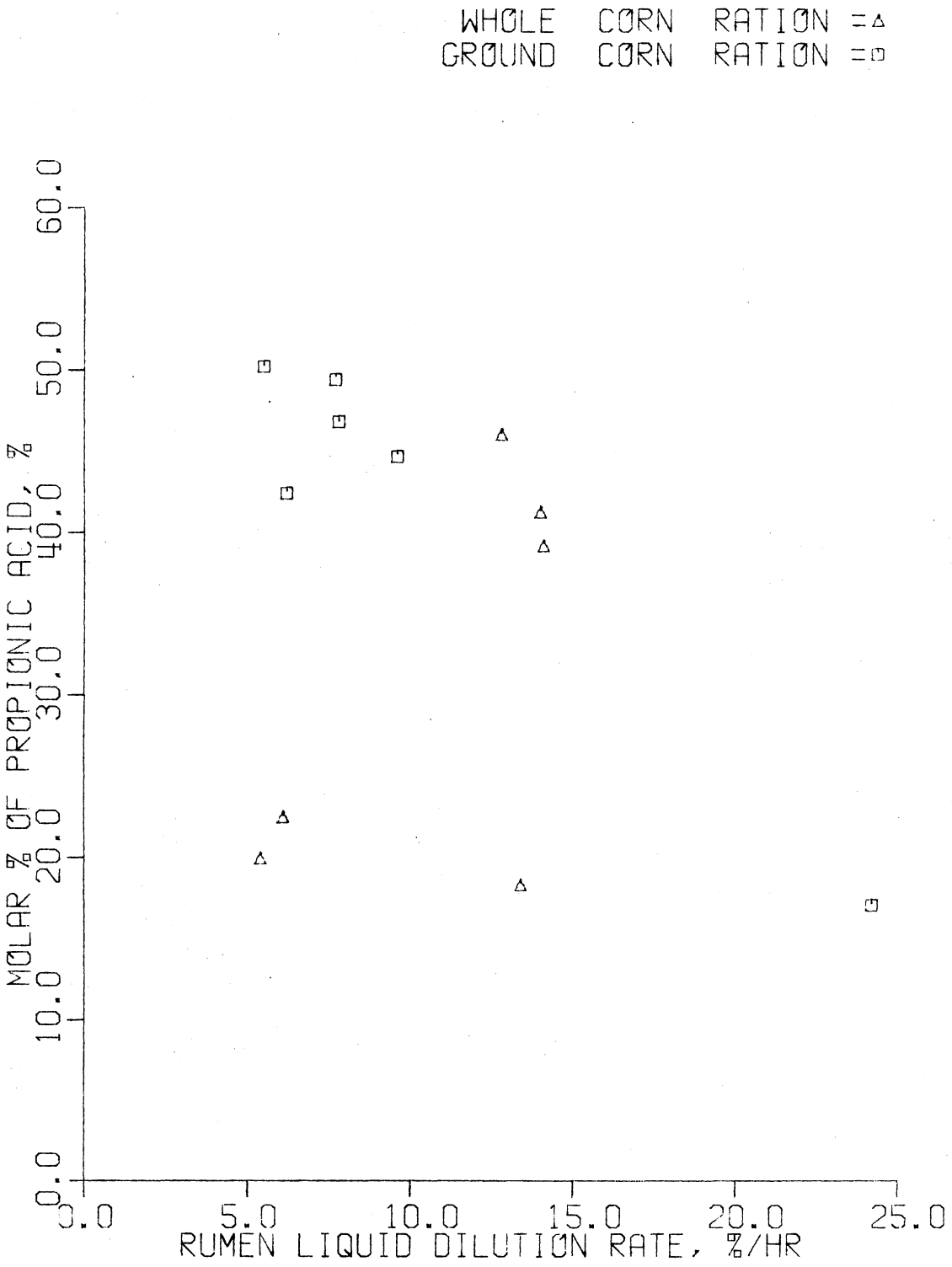


TABLE XXXIV

RATIO OF 2-METHYLBUTYRIC ACID TO 3-METHYLBUTYRIC ACID IN THE RUMEN FLUID OF STEERS CONSUMING EITHER A WHOLE OR GROUND CORN HIGH-CONCENTRATE RATION

Animal	Diet	Expt	$\frac{2MB}{3MB}$[a]	s[b]
608	WC	Ac	2.89	0.28
608	WC	Pr	1.55	0.34
608	WC	Bu	1.81	0.14
422	WC	Ac	1.37	0.21
422	WC	Pr	0.61	0.08
422	WC	Bu	3.43	0.28
612	GC	Ac	0.88	0.04
612	GC	Pr	2.24	0.05
612	GC	Bu	1.39	0.22
608	GC	Ac	1.95	0.31
608	GC	Pr	1.88	0.11
608	GC	Bu	5.20	0.19

[a]Ratio of 2-methylbutyric acid to 3-methylbutyric acid.

[b]Standard deviation of two observations for each mean.

discussed by Allison and Bryant (1963), Allison (1969) and Sauer et al. (1975). Traditionally, the presence of 2MB and 3MB acids in rumen fluid has been attributed to the catabolism of isoleucine (ile) and leucine (leu), respectively. In contrast, Clifford and Tillman (1968) found that 2MB plus 3MB acids accounted for approximately 1% of the total VFA concentration in the rumen fluid of sheep fed a purified diet containing urea as the only nitrogen source. Bacteria lysis and subsequent degradation of bacterial amino acids could have been responsible for this observation. Evidence presented by Allison et al. (1966) and, more recently, by Sauer et al. (1975) indicates that de novo synthesis of these branched VFA's does occur.

Comparison of the observed 2MB:3MB ratios (Table XXXIV) to the ile:leu ratios of the corn diet and that normally found in bacterial protein (Table XXXV) suggests that the metabolism of these branched-chain VFA is not strictly related to the ratios of the respective amino acids in the substrate (diet) and product (bacterial protein). Differences could be attributed to differential rates of amino acid degradation and synthesis as well as differential rates of de novo synthesis of these branched-chain VFA's. Alternately, an elevated de novo production of 2MB relative to 3MB could explain the observed increase in 2MB:3MB as compared to ile:leu present in the feed. Elucidation of the mechanisms involved should be investigated.

Figure 42 illustrates a curious relationship between the rumen liquid turnover rate constant and the 2MB:3MB ratio. The 2MB:3MB ratio tended to increase as rumen dilution rate increased. It is tempting to suggest that an increase in the 2MB:3MB ratio is associated with an increased bacterial growth rate. Surprisingly, the 608-GC-Bu data

TABLE XXXV

RATIO OF ISOLEUCINE TO LEUCINE IN THE WHOLE AND GROUND CORN RATION AS COMPARED TO VALUES REPORTED TO OCCUR IN RUMEN BACTERIA

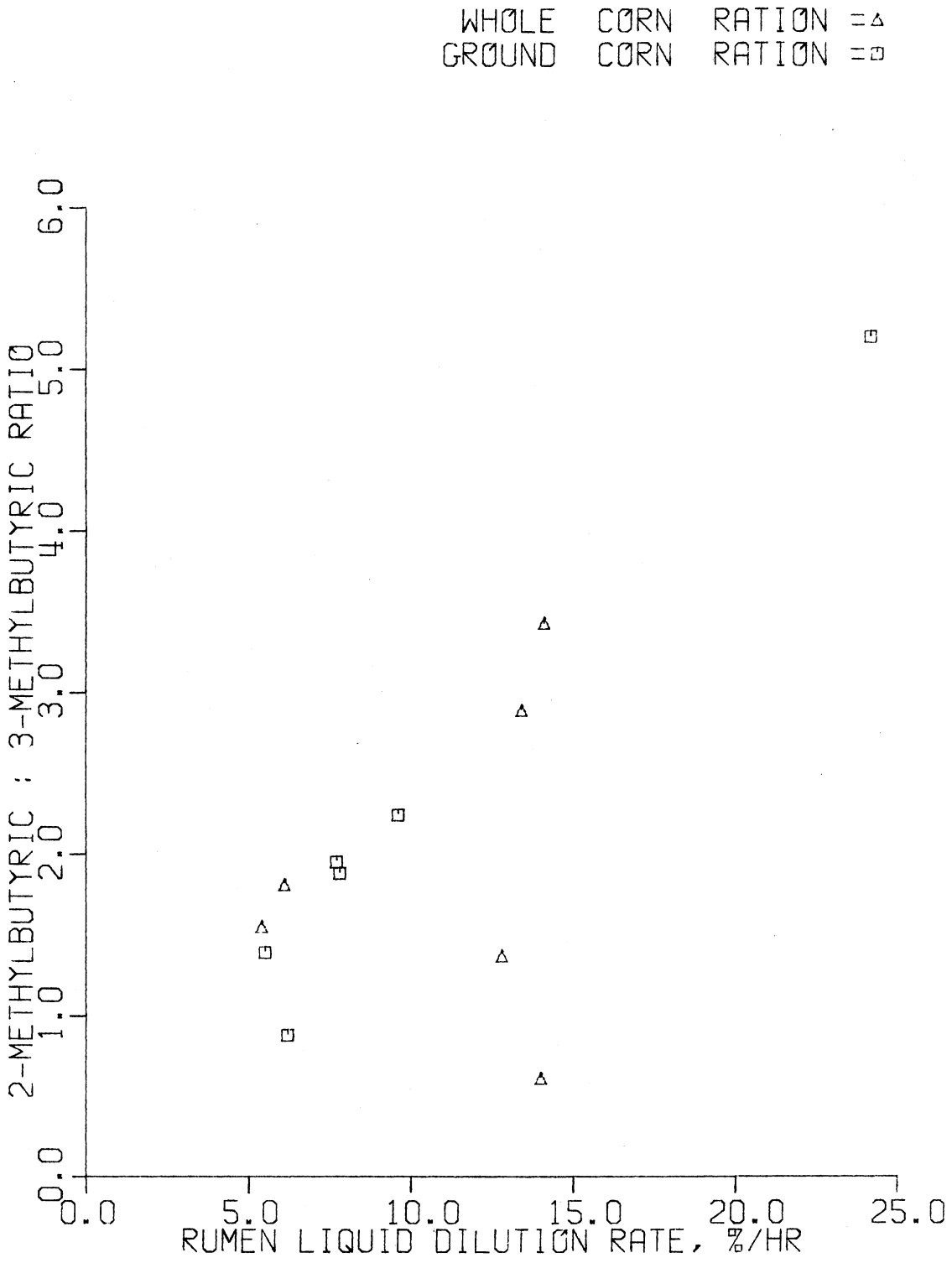
Item	$\frac{ile}{leu}$[a]
Diet[b]	0.49
Bacteria	
Hungate (1966)	0.80
Purser and Buechler (1966)	0.88
Sauer et al. (1975)	0.64

[a]Ratio of isoleucine to leucine.

[b]Calculated from values shown in Table X and standard feed composition tables.

Figure 42. Relationship Between the Ratio of 2-Methylbutyrate to 3-Methylbutyrate and the Dilution Rate of the Rumen Liquid Phase.

2-MB : 3-MB RATIO VS RUMEN DILUTION RATE



seems to agree with this trend. It has been demonstrated that certain species of rumen bacteria require branched-chain VFA's for growth (Bentley et al., 1954). Furthermore, an increase in the growth rate of a bacterial population is associated with an increase in dilution rate (Stouthamer and Bettenhaussen, 1973). The biochemical mechanisms and relationships associated with branched-chain VFA metabolism and bacterial growth should be investigated.

VFA Pool Size. The quantity of material in each extracellular VFA compartment can be estimated according to the relationship

$$Q_{ik} = C_{ik} V_k \qquad (5.3)$$

where Q_{ik} is the amount of material in the i^{th} compartment during the k^{th} experiment, C_{ik} is the mean concentration of the i^{th} VFA during the k^{th} infusion and V_k is the rumen liquid volume during the k^{th} infusion experiment. These data are presented in Table XXXVI and are summarized according to each animal-ration period and ration type in Table XXXVII. Generally, the VFA pool sizes paralleled the VFA concentrations; however, the acetate pool size was similar for both the ground and whole corn diets. Steers consuming GC tended ($P < .10$) to have a higher propionate pool size and a lower butyrate pool size than did steers consuming the WC diet.

Some of the differences in VFA pool size within a period may be partly explained by different levels of feed intake as seen from the relationship in Figure 43. There was a tendency for higher VFA pool sizes at higher levels of dry matter intake.

TABLE XXXVI

EXTRACELLULAR VFA COMPARTMENT SIZE FOR EACH INFUSION EXPERIMENT[a]

Animal	Diet	Expt	Q_a[b]		Q_p		Q_b		Q_v	
			moles	gac	moles	gac	moles	gac	moles	gac
608	WC	Ac	3.30	6.60	1.05	3.14	0.84	3.37	0.16	0.80
608	WC	Pr	1.90	3.79	0.66	1.98	0.48	1.92	0.06	0.28
608	WC	Bu	1.76	3.52	0.68	2.03	0.36	1.43	0.06	0.31
422	WC	Ac	1.57	3.14	2.17	6.52	0.75	3.00	0.12	0.61
422	WC	Pr	1.61	3.22	2.03	6.08	0.95	3.81	0.17	0.85
422	WC	Bu	1.86	3.72	2.16	6.49	1.15	4.62	0.16	0.81
612	GC	Ac	1.63	3.27	1.79	5.37	0.47	1.88	0.09	0.43
612	GC	Pr	3.16	6.32	3.26	9.77	0.57	2.26	0.13	0.65
612	GC	Bu	1.75	3.50	2.32	6.97	0.38	1.54	0.08	0.38
608	GC	Ac	1.59	2.32	1.58	4.75	0.33	1.32	0.05	0.25
608	GC	Pr	2.20	4.40	2.63	7.88	0.51	2.02	0.11	0.57
608	GC	Bu	2.24	4.49	0.68	2.05	0.84	3.38	0.10	0.48

[a]Compartment size expressed in both moles and gram-atoms of carbon (gac).

[b]a - acetate, p - propionate, b - butyrate, v - valerate.

TABLE XXXVII

MEAN EXTRACELLULAR VFA COMPARTMENT SIZE FOR EACH ANIMAL-RATION PERIOD

Period	n	Pool Size (gac)			
		Q_a	Q_p	Q_b	Q_v
608-WC	3	4.64	2.38[a]	2.24	0.46
422-WC	3	3.36	6.36[b]	3.81	0.76
612-GC	3	4.36	7.37	1.89	0.49
608-GC	3	3.74	4.89	2.24	0.43
608-GC[e]	2	3.36	6.32	1.67	0.41
WC	6	4.00	4.37[c]	3.03[c]	0.61
GC	6	4.05	6.13	2.07	0.46
GC[e]	5	3.96	6.95[d]	1.80[d]	0.46
WC SE	6	0.55	1.01	0.40	0.09
GC SE[e]	6	0.57	0.87	0.38	0.09
GC SE[e]	5	0.63	0.95	0.41	0.10

[a,b]Comparisons between animals within a ration type with different subscripts are different ($P < .05$).

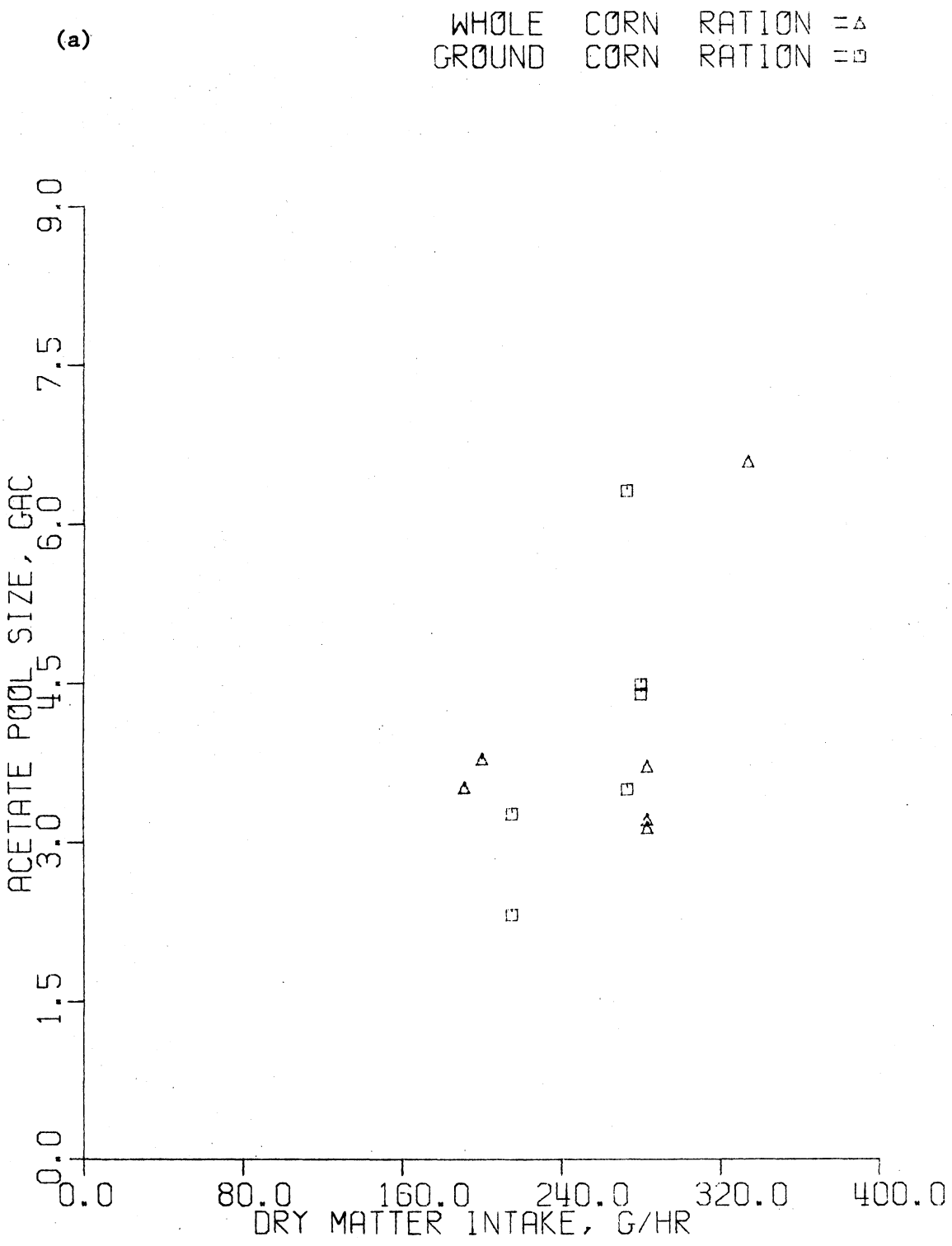
[c,d]Comparisons between whole and ground corn with different superscripts tended to be different ($P < .10$).

[e]The 608-GC-Bu data was excluded from calculations.

Figure 43. Relationship Between VFA Pool Size and Dry Matter Intake. (a) Acetate pool, (b) Propionate pool, (c) Butyrate pool.

ACETATE POOL SIZE VS DRY MATTER INTAKE

(a)



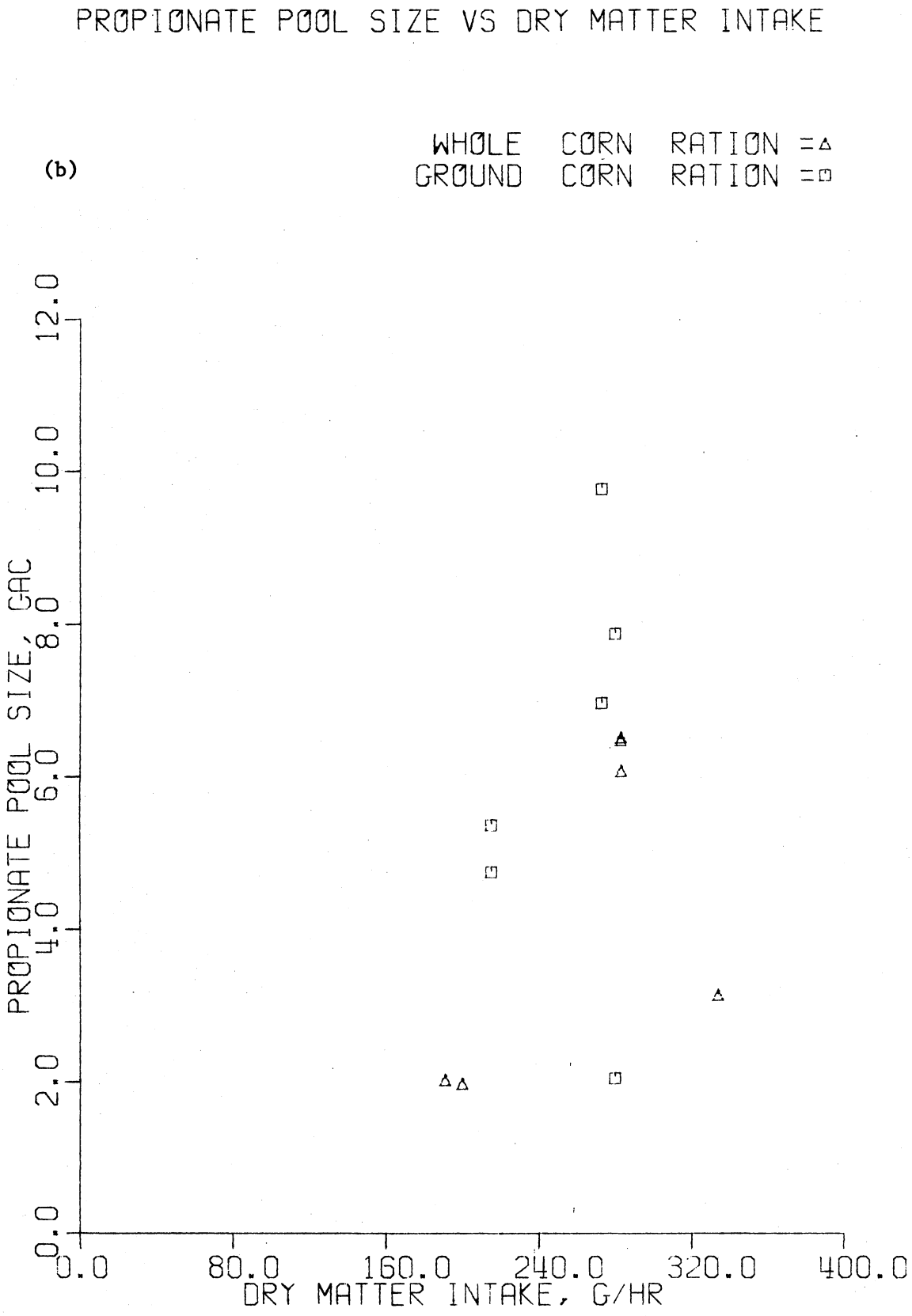


Figure 43. (Continued)

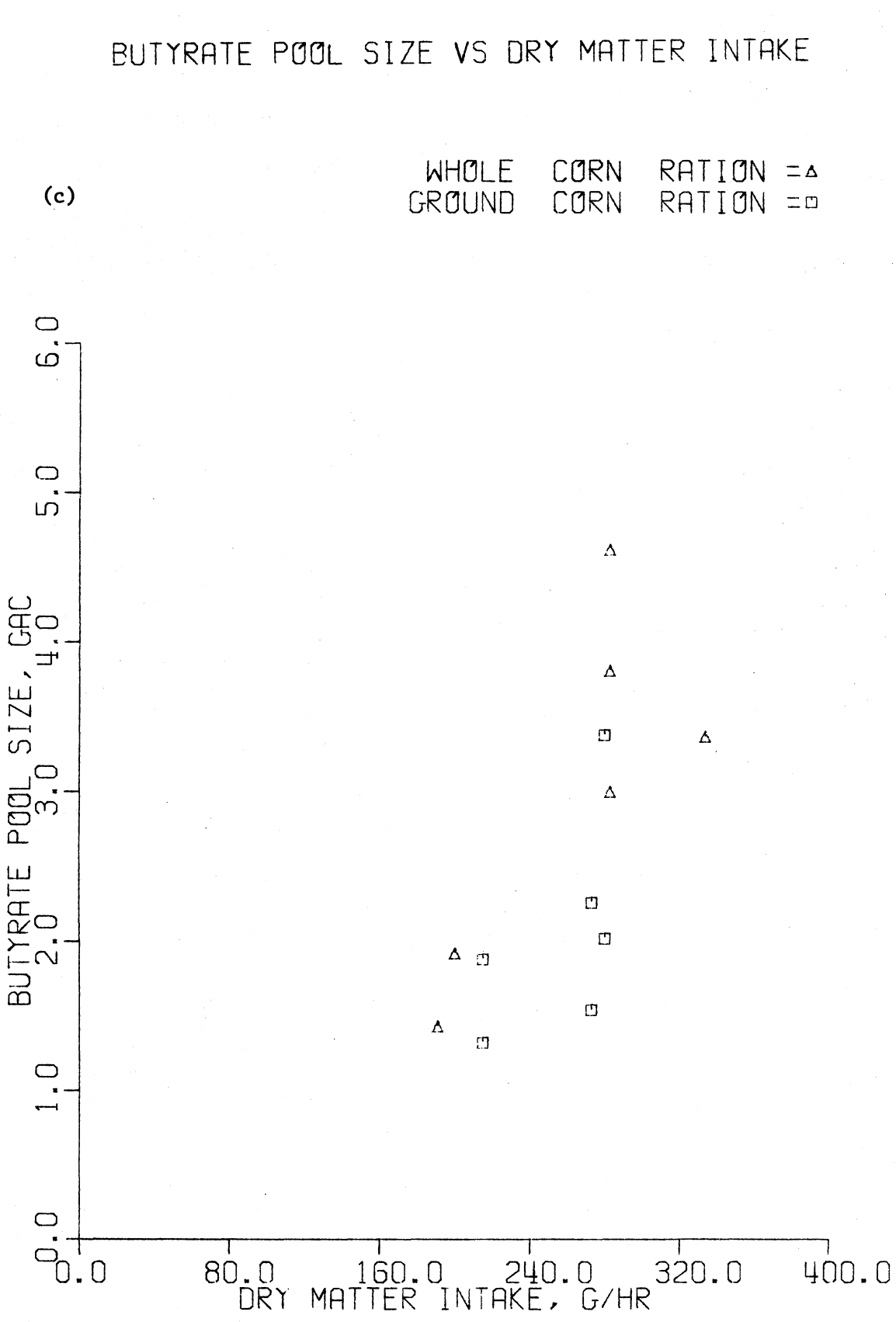


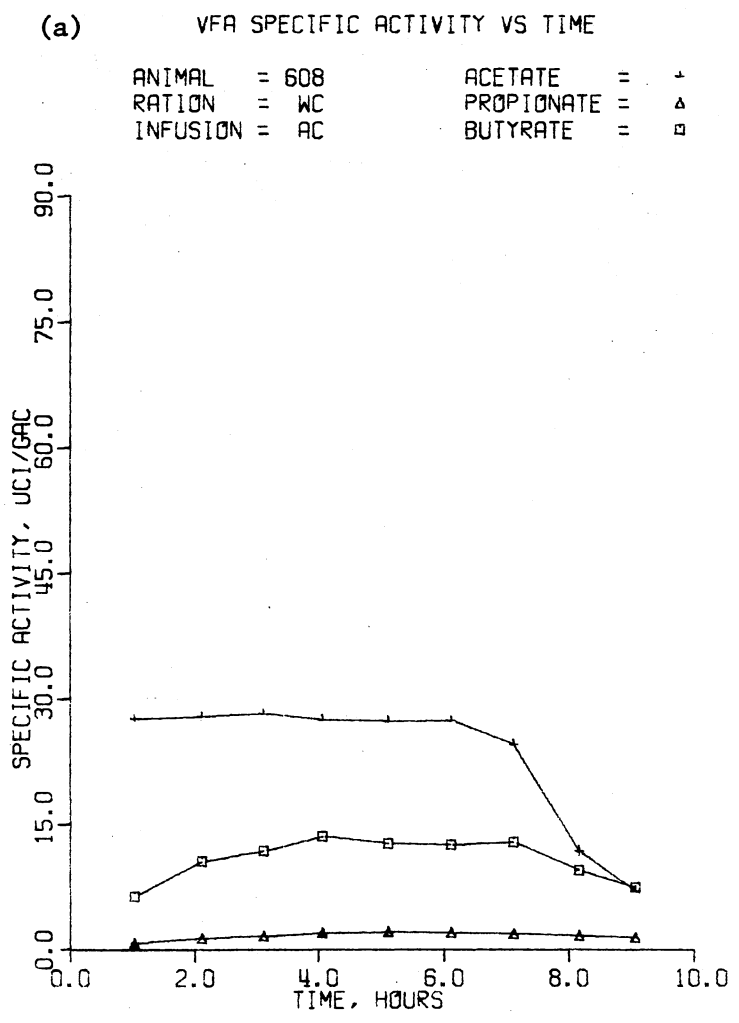
Figure 43. (Continued)

VFA Specific Activity. The priming dose and rate of ^{14}C-VFA infusion during each experiment was shown previously in Table XIV. The specific activities (uncorrected for infusion rate, animal weight and feed rate) of acetate, propionate, butyrate and valerate during each infusion experiment are presented in Tables LV-LVIII, Appendix E and in Figures 44-47. The specific activity (μCi/gac) of the infused VFA compartment, as well as the other VFA compartments, appeared to reach a steady-state level during at least the last two hours of all infusion periods. Consequently, the steady-state specific activity of each acid during each infusion was estimated from the 4, 5 and 6 or 5 and 6 hour samples. These values were corrected to an infusion rate of 2.0 μCi/min and are presented in Table XXXVIII.

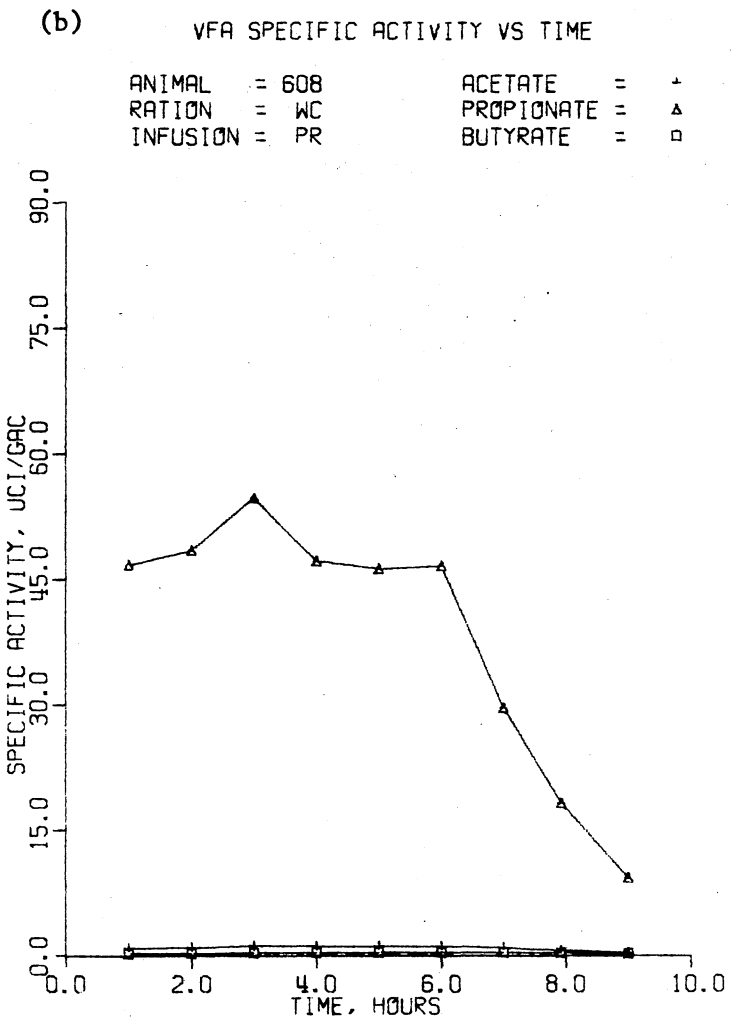
Shipley and Clark (1972) suggested that when animals differ in body weight, specific activity data should be adjusted to an arbitrary standard animal weight in order to make the comparisons valid. This is founded on the principle that larger animals tend to have larger metabolic pools (compartments) and consequently would dilute a given amount of tracer more than a smaller animal. Likewise, when studying metabolic pools within the digestive tract of fed animals, such as the rumen VFA's, animals with higher levels of feed intake probably have larger metabolic pools than animals consuming the same feed, but at a lower level of intake. Therefore, the steady-state specific activity data in Table XXXVIII was corrected to a standard steer weight (SSW) of 400 kg and a standard feed rate (SFR) of 300 g/hr according to the equation

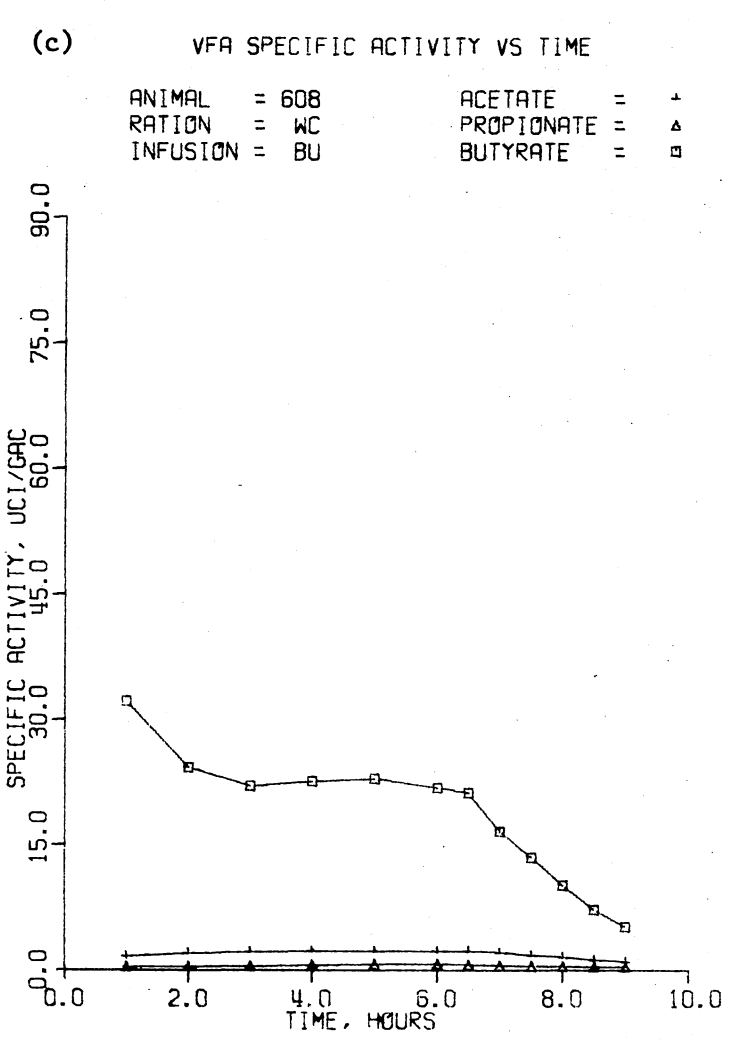
Figure 44. Specific Activity of the VFA's for Animal 608 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment, (d) Specific activity of valerate during the acetate and propionate experiment.

(a)


VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 608
RATION = WC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
15.0
30.0
45.0
60.0
75.0
90.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)


VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 608
RATION = WC
INFUSION = PR
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
15.0
30.0
45.0
60.0
75.0
90.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS



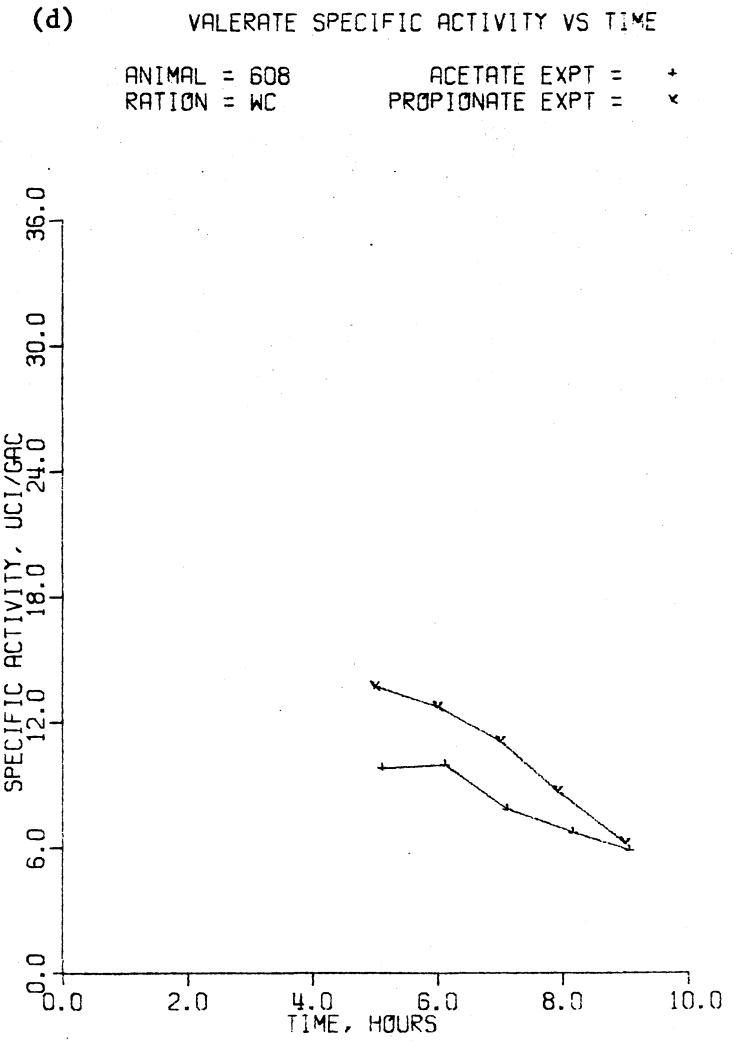
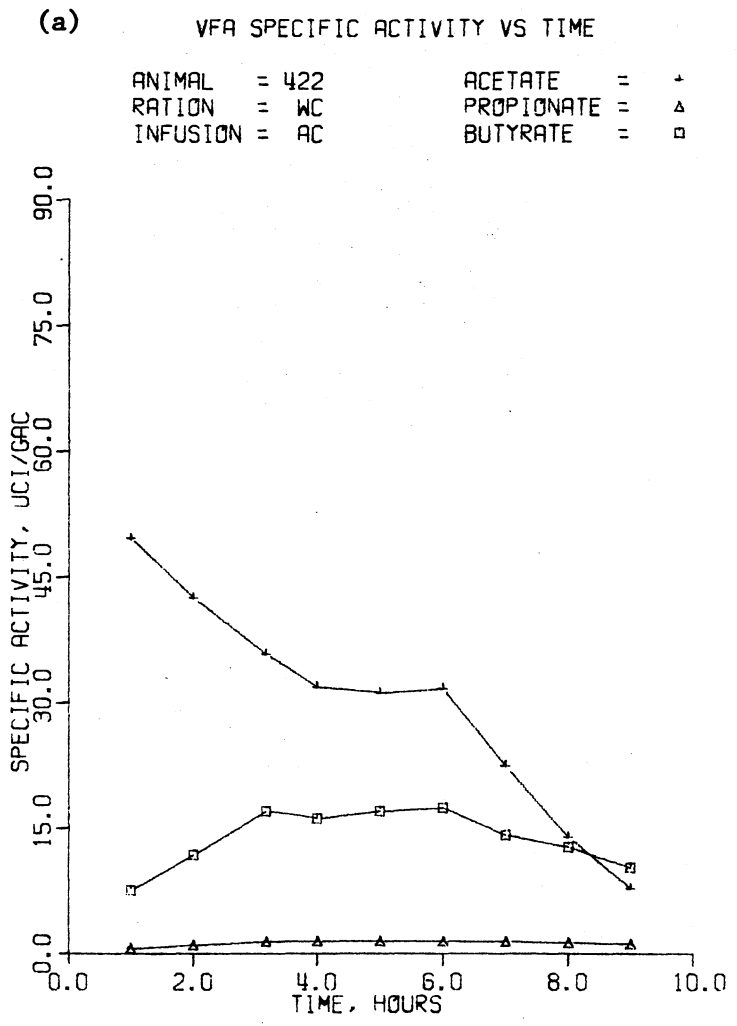


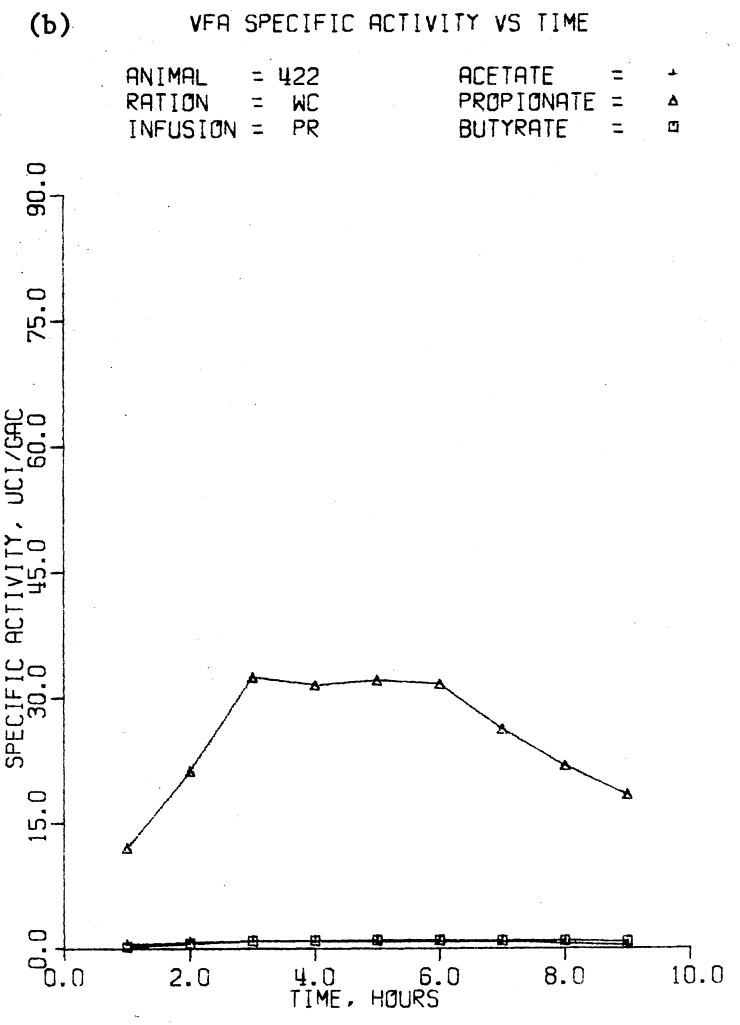
Figure 44. (Continued)

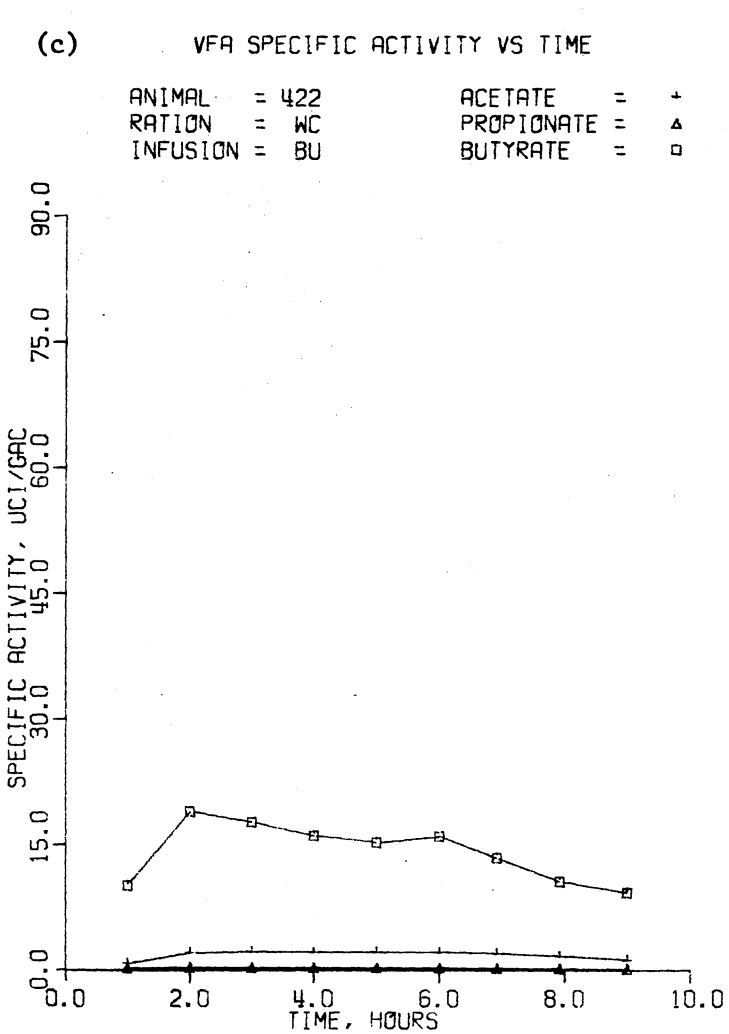
Figure 45. Specific Activity of the VFA's for Animal 422 Consuming the Whole Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment, (d) Specific activity of valerate during the acetate and propionate experiment.

(a)


VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 422
RATION = WC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
15.0
30.0
45.0
60.0
75.0
90.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)


VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 422
RATION = WC
INFUSION = PR
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
15.0
30.0
45.0
60.0
75.0
90.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS



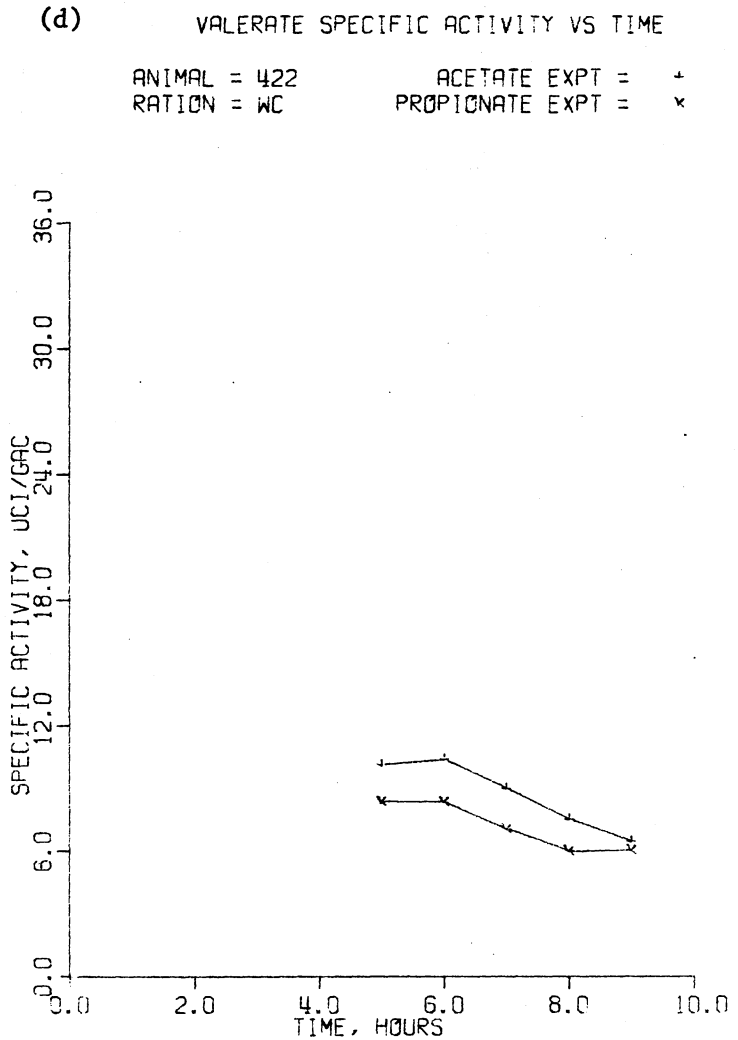
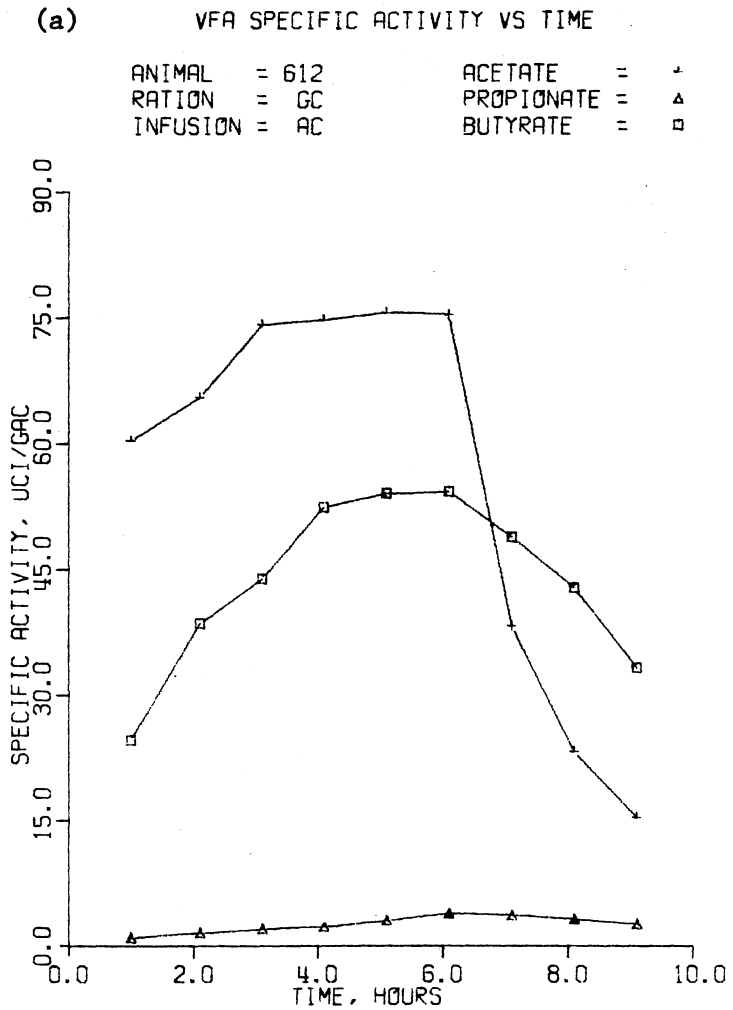


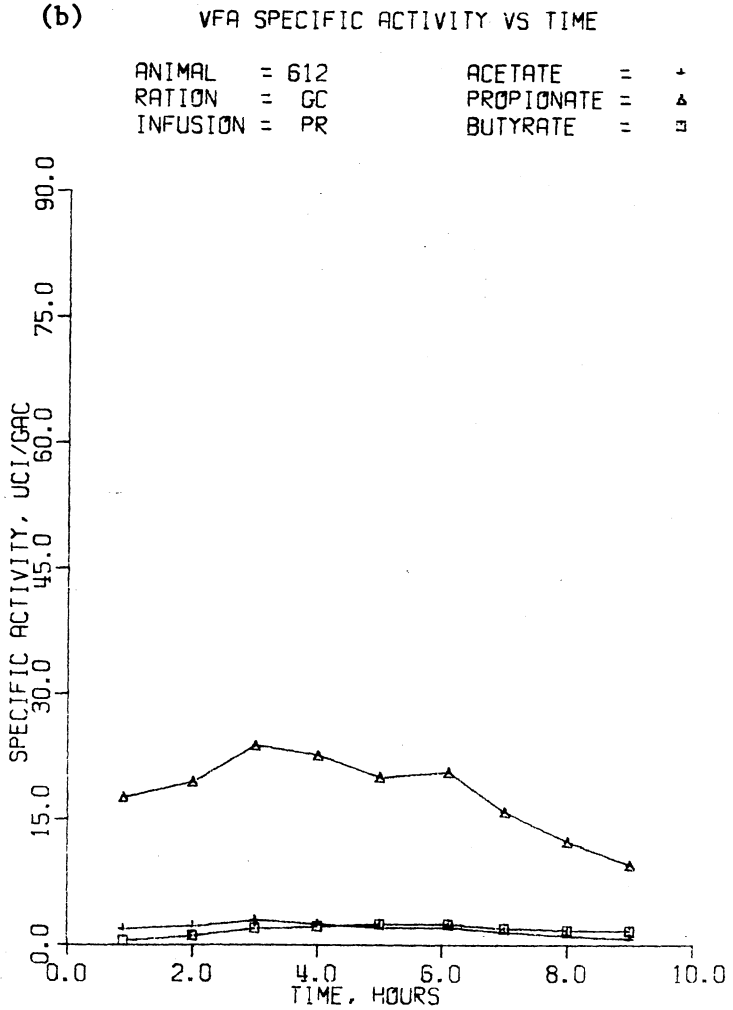
Figure 45. (Continued)

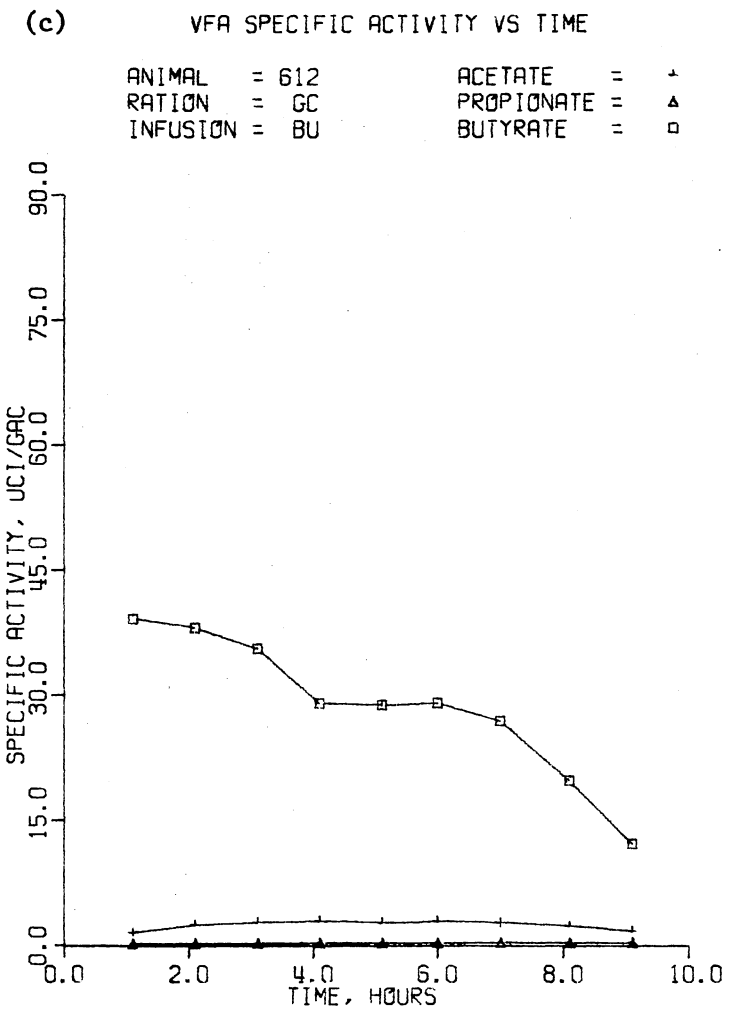
Figure 46. Specific Activity of the VFA's for Animal 612 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment, (d) Specific activity of valerate during the acetate and propionate experiments.

(a)


VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 612
RATION = GC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
15.0
30.0
45.0
60.0
75.0
90.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)


VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 612
RATION = GC
INFUSION = PR
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
15.0
30.0
45.0
60.0
75.0
90.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS



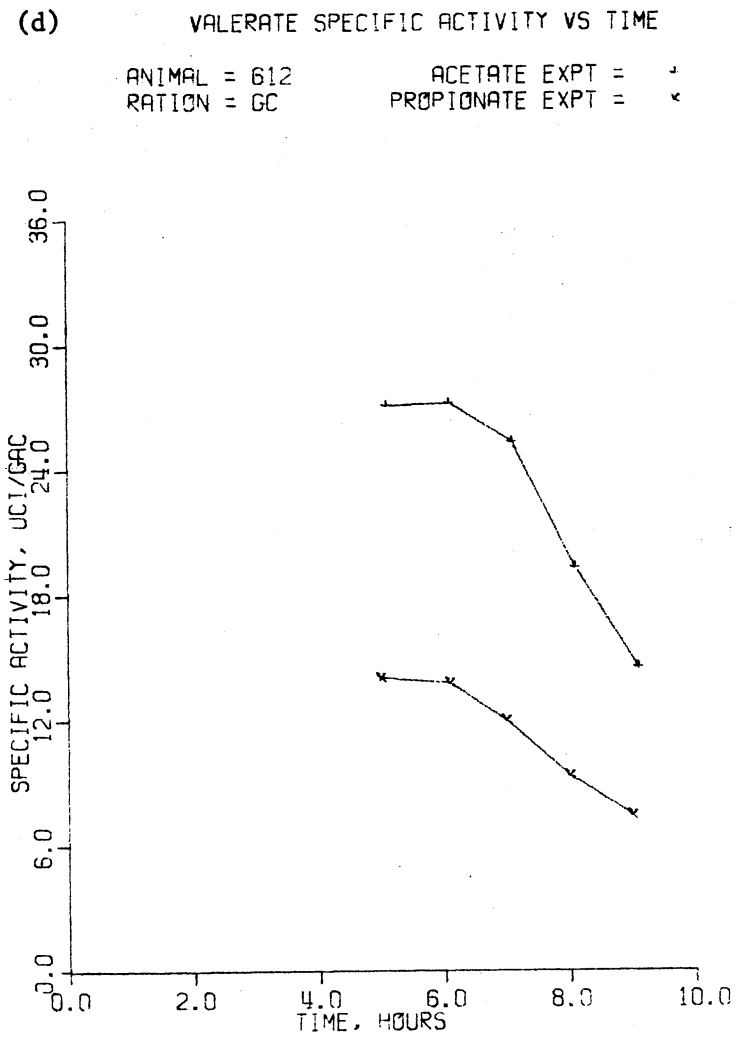
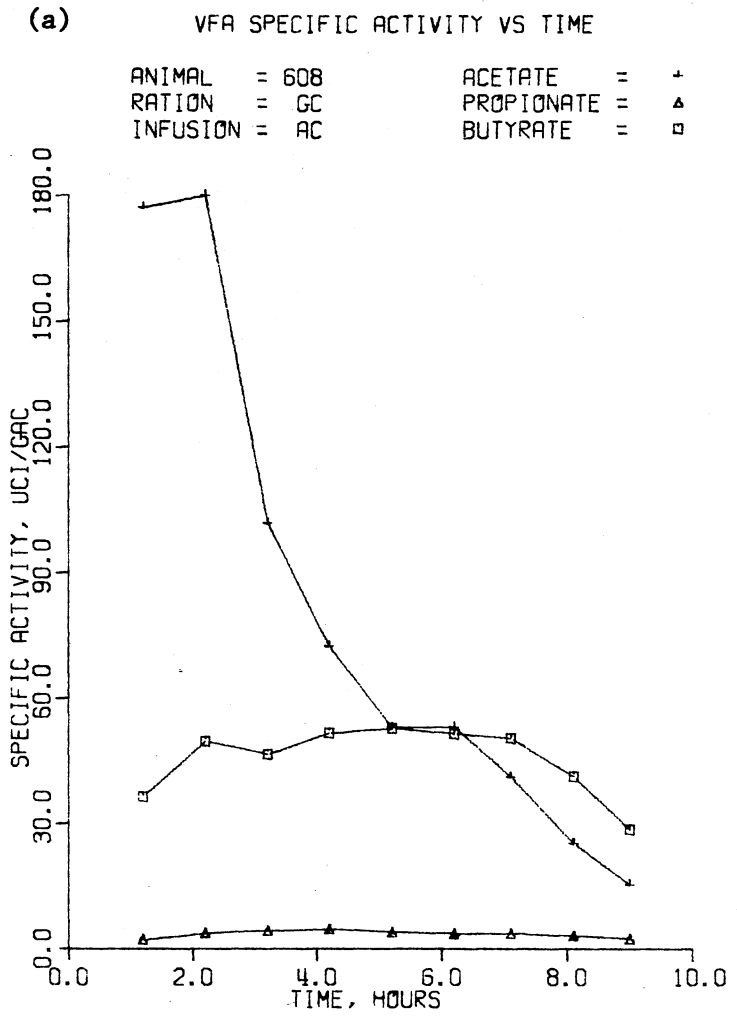
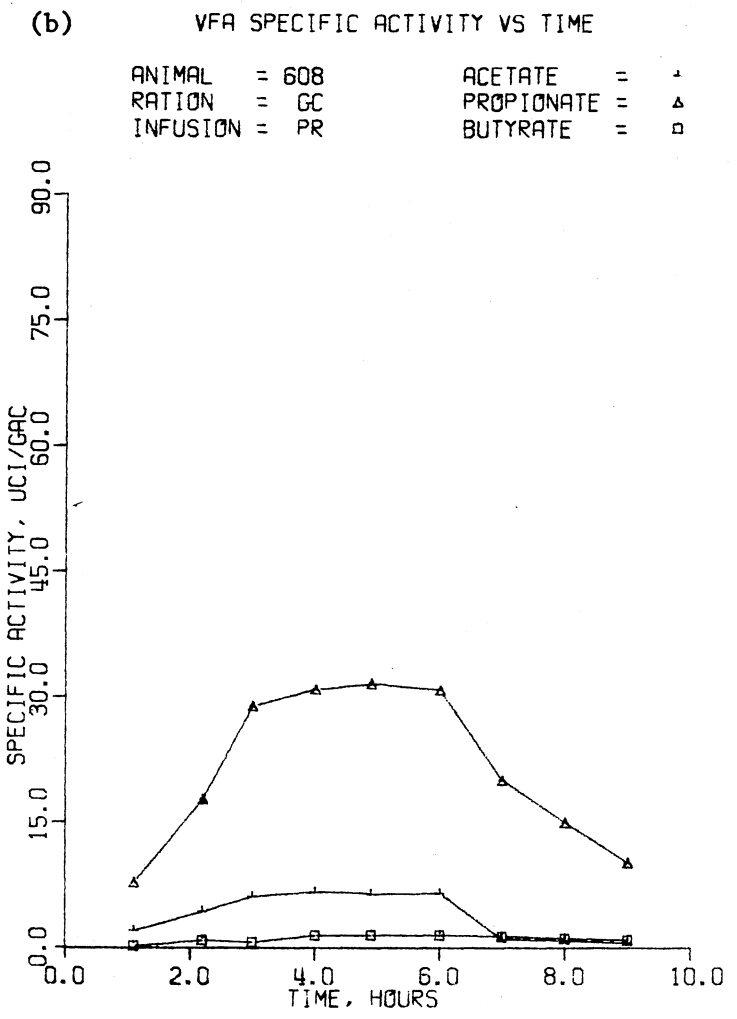
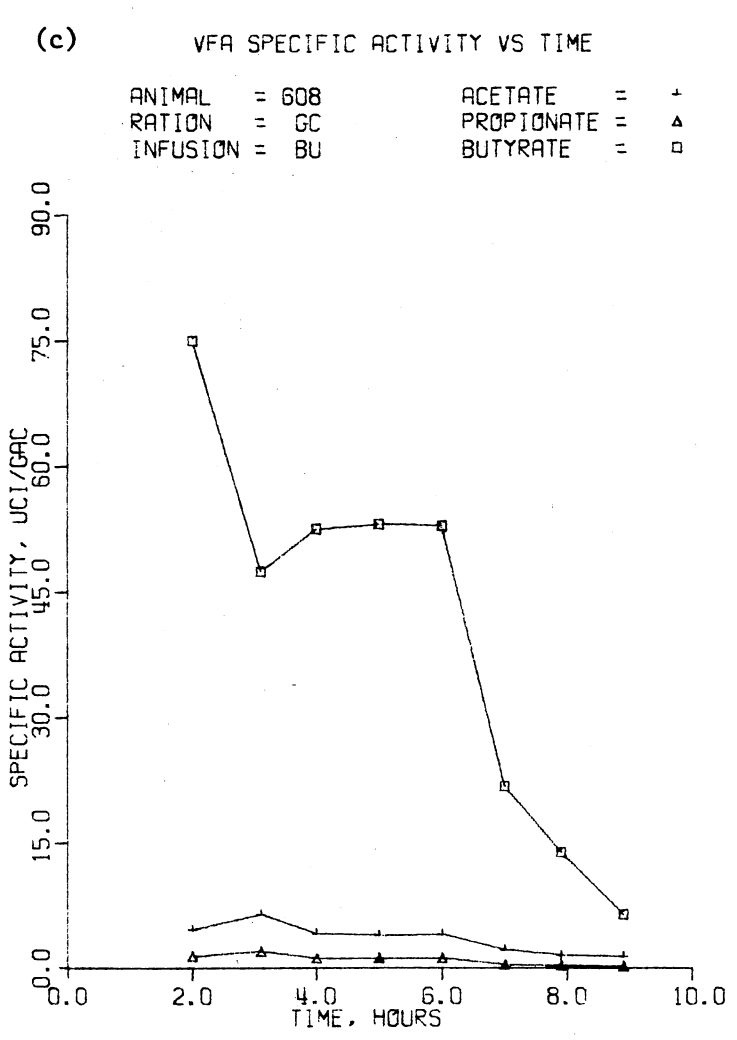


Figure 46. (Continued)

Figure 47. Specific Activity of the VFA's for Animal 608 Consuming the Ground Corn Diet. (a) Acetate experiment, (b) Propionate experiment, (c) Butyrate experiment, (d) Specific activity of valerate during the acetate and propionate experiments.


(a)
VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 608
RATION = GC
INFUSION = AC
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
30.0
60.0
90.0
120.0
150.0
180.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS


(b)
VFA SPECIFIC ACTIVITY VS TIME
ANIMAL = 608
RATION = GC
INFUSION = PR
ACETATE =
PROPIONATE =
BUTYRATE =
SPECIFIC ACTIVITY, UCI/GAC
0.0
15.0
30.0
45.0
60.0
75.0
90.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS



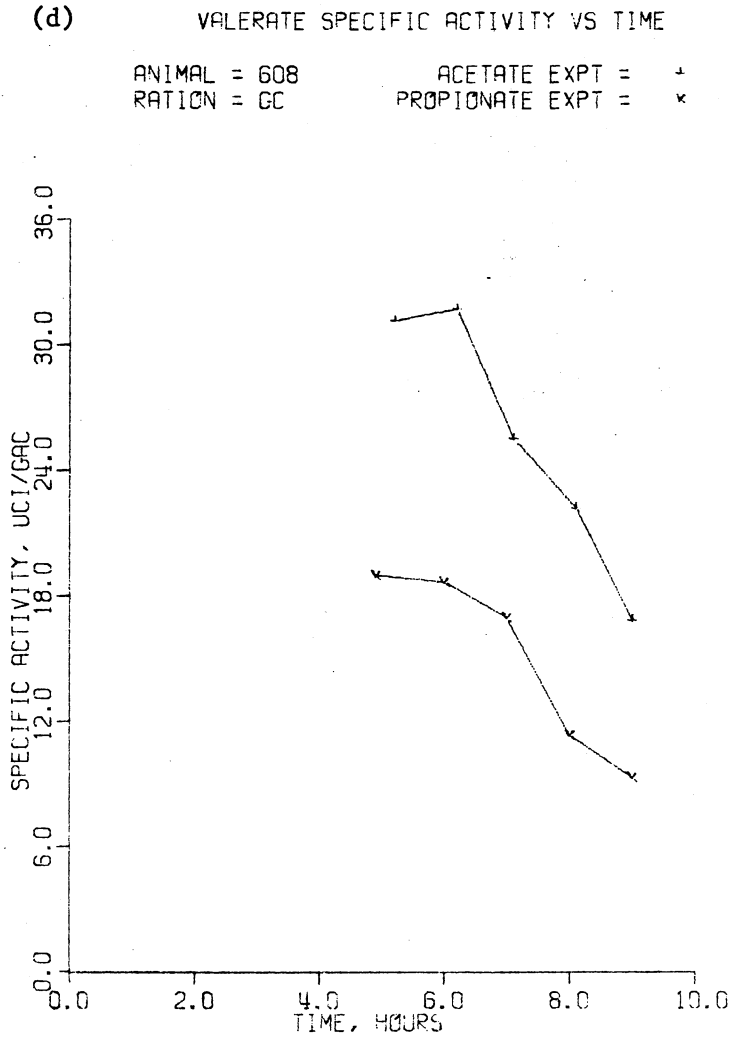


Figure 47. (Continued)

TABLE XXXVIII

STEADY-STATE SPECIFIC ACTIVITY OF RUMEN VFA CORRECTED FOR DIFFERENCES IN INFUSION RATE

Animal	Diet	Expt	n	Specific Activity (μCi/gac)[a]			
				a_{ae}	a_{pe}	a_{be}	a_{ve}
608	WC	Ac	3	37.5 ±1.04	2.74±0.201	17.2 ±0.56	13.5±0.44
608	WC	Pr	2	2.01±0.19	87.6 ±1.62	0.62±0.12	25.0±1.32
608	WC	Bu	3	9.60±0.64	2.90±0.31	95.6 ±3.28	-
422	WC	Ac	3	43.2 ±1.23	1.95±0.14	23.6 ±0.61	14.0±0.46
422	WC	Pr	3	1.44±0.17	57.1 ±1.45	1.67±0.16	15.1±0.04
422	WC	Bu	3	9.22±0.77	0.92±0.14	68.2 ±1.75	-
612	GC	Ac	3	79.7 ±2.57	3.63±0.63	57.3 ±1.90	28.8±0.97
612	GC	Pr	2	4.10±0.31	41.2 ±0.60	4.97±0.44	28.2±0.75
612	GC	Bu	2	14.5 ±0.81	1.38±0.18	147.3 ±4.36	-
608	GC	Ac	2	55.7 ±1.97	3.91±0.17	54.6 ±1.37	33.1±0.82
608	GC	Pr	3	11.7 ±0.91	56.1 ±1.92	2.64±0.31	33.9±0.82
608	GC	Bu	3	17.0 ±0.80	4.69±0.35	225.2 ±6.30	-

[a]Specific activity adjusted to an infusion rate of 2.0 μCi/min. Values represent the steady-state specific activity ± standard deviation.

$$a_{ike} = a'_{ike} \times \frac{SW_k}{400} \times \frac{FR_k}{300} \qquad (5.4)$$

where a_{ike} is the steady-state specific activity of the i^{th} acid during the k^{th} experiment corrected for differences in infusion rate, steer weight and feed rate, a'_{ike} is the steady-state specific activity corrected for differences in infusion rate (from Table XXXVIII), SW_k is the weight (kg) of the steer during the k^{th} experiment and FR_k is the feed rate (g/hr) during the k^{th} experiment. The tabulated values of a_{ike} are presented in Table XXXIX, while the ration means are given in Table XL. These values were used in the calculation of turnover rates associated with compartment models describing VFA production and interconversion.

Experimental difficulties prevented the fractionation of 2-methylbutyric acid and 3-methylbutyric acid from the preparative gas chromatograph 'isovaleric' fraction. Therefore, a determination of the specific activity of each isomer was not possible. The specific activity of the 'isovaleric' fraction was calculated and is given in Tables LV-LVIII, Appendix E.

The quantity of tracer in each VFA compartment during each infusion experiment was estimated from the equation

$$q_{ik} = a'_{ike} Q_{ik} \qquad (5.5)$$

where q_{ike} is the steady-state total quantity of ^{14}C radioactivity (μCi) in compartment i during the k^{th} experiment, a'_{ike} is the specific activity (μCi/gac) corrected for infusion rate (Table XXXVIII) and Q_{ik} is the compartment size (gac) from Table XXXVI. Values for q_{ike}

TABLE XXXIX

STEADY-STATE SPECIFIC ACTIVITY OF RUMEN VFA CORRECTED FOR DIFFERENCES IN INFUSION RATE, STEER WEIGHT AND FEED RATE

Animal	Diet	Expt	n	Specific Activity (μCi/gac)[a]			
				a_{ae}	a_{pe}	a_{be}	a_{ve}
608	WC	Ac	3	39.8 ±1.11	2.91±0.21	18.3 ±0.59	14.4±0.46
608	WC	Pr	2	1.35±0.13	59.0 ±1.09	0.42±0.09	16.8±0.89
608	WC	Bu	3	6.04±0.41	1.83±0.19	60.1 ±2.06	-
422	WC	Ac	3	38.0 ±1.09	1.71±0.12	20.7 ±0.54	12.4±0.41
422	WC	Pr	3	1.31±0.15	52.0 ±1.32	1.52±0.14	13.7±0.03
422	WC	Bu	3	8.24±0.69	0.83±0.13	61.0 ±1.57	-
612	GC	Ac	3	65.8 ±2.12	3.00±0.52	47.4 ±1.57	23.8±0.80
612	GC	Pr	2	4.18±0.31	42.0 ±0.61	5.06±0.45	28.7±0.76
612	GC	Bu	2	14.9 ±0.84	1.43±0.19	151.8 ±4.49	-
608	GC	Ac	2	35.0 ±1.24	2.46±0.11	34.3 ±0.86	20.8±0.52
608	GC	Pr	3	9.96±0.77	47.6 ±1.63	2.25±0.26	28.8±0.69
608	GC	Bu	3	14.1 ±0.66	3.89±0.29	187.1 ±5.24	-

[a]Specific activity adjusted to an infusion rate of 2.0 μCi/min, standard steer weight (SSW) of 400 kg and standard feed rate (SFR) g 300 g DM/hr. Values represent the steady-state specific activity ± standard deviation.

TABLE XL

MEAN STEADY-STATE SPECIFIC ACTIVITY OF RUMEN VFA CORRECTED FOR DIFFERENCES IN INFUSION RATE, STEER WEIGHT AND FEED RATE

Diet	Expt	Specific Acitvity (μCi/gac)[a]			
		$^{a}_{ae}$	$^{a}_{pe}$	$^{a}_{be}$	$^{a}_{ve}$
WC	Ac	38.9 ± 0.91	2.31±0.60	19.5 ±1.20	13.4±1.00
WC	Pr	1.33± 0.02	55.5 ±3.49	0.97±0.55	15.3±1.55
WC	Bu	7.14± 1.10	1.33±0.50	60.6 ±0.44	--
GC	Ac	50.4 ±15.4	2.73±0.27	40.8 ±6.52	22.3±1.50
GC	Pr	7.07± 2.89	44.8 ±2.80	3.65±1.41	28.8±0.05
GC	Bu	14.5 ± 0.40	2.66±1.23	170.0 ±17.7	-

[a]Average across diet of the corrected specific activity presented in Table XXXIX. Values are means ± standard error of the mean (n=2).

are already corrected for differences in Q_{ik} due to steer weight and feed rate based on reasoning presented earlier. Tabulated values for q_{ike} are presented in Table XLI, while the ration means are given in Table XLII. These values will be used later in determining the turnover rate constants associated with compartment models describing ruminal VFA production and interconversion.

The transfer of carbon from one VFA to another is described by the transfer ratio

$$Y_{ji} = \frac{a_{je}}{a_{ie}} \tag{5.6}$$

where Y_{ji} is the transfer ratio of carbon from compartment i to compartment j, a_{je} is the steady-state specific activity of the j^{th} compartment and a_{ie} is the steady-state specific activity of the i^{th} compartment (the one being infused). The ^{14}C-transfer ratios for each animal-ration period are presented in Table XLIII, while the mean ^{14}C-transfer ratios for each ration are listed in Table XLIV.

Both steers consuming the WC ration had similar (46 vs 55%) acetate to butyrate transfer ratios, while the difference between the steers consuming the GC ration was much larger. During the 608-GC period essentially all the butyrate carbon was derived from extracellular acetate in Animal 608. Steers consuming WC appeared to have a lower Y_{ba} when compared to steers consuming GC. Only 50.3% of the butyrate carbon was derived from acetate carbon on the WC ration, while on the GC ration 85.0% of the butyrate carbon came from acetate carbon.

Considering the model in Figure 11, synthesis of extracellular

TABLE XLI

STEADY-STATE TOTAL RADIOACTIVITY OF RUMEN VFA DURING EACH INFUSION EXPERIMENT

Animal	Diet	Expt	n	Total Radioactivity (μCi)[a]		
				q_{ae}	q_{pe}	q_{be}
608	WC	Ac	3	247.0 ±11.3	8.60± 0.74	58.1 ± 3.05
608	WC	Pr	2	7.61± 0.90	173.0 ±16.1	1.19± 0.24
608	WC	Bu	3	33.8 ± 2.69	5.90± 0.73	137.0 ± 8.97
422	WC	Ac	3	136.0 ±27.1	12.7 ± 1.38	70.7 ± 5.72
422	WC	Pr	3	4.63± 0.88	347.0 ±55.5	6.35± 1.28
422	WC	Bu	3	34.3 ± 4.46	6.00± 1.15	315.0 ±29.8
612	GC	Ac	3	261.0 ±27.6	19.5 ± 3.88	108.0 ±12.0
612	GC	Pr	2	25.9 ± 3.88	403.0 ±65.4	11.2 ± 2.63
612	GC	Bu	2	50.6 ± 4.95	9.65± 1.45	226.8 ±25.9
608	GC	Ac	2	129.0 ±24.4	18.6 ± 3.27	72.0 ±13.8
608	GC	Pr	3	51.6 ± 8.93	441.7 ±72.2	5.34± 0.76
608	GC	Bu	3	76.2 ± 5.05	9.61± 1.07	761.0 ±83.8

[a]Values are the mean ± standard deviation. See text for discussion as to how values were calculated.

TABLE XLII

MEAN STEADY-STATE TOTAL RADIOACTIVITY OF RUMEN VFA IN STEERS CONSUMING EITHER A WHOLE OR GROUND CORN HIGH-CONCENTRATE DIET

Diet	Expt	Total Radioactivity (μCi)[a]		
		q_{ae}	q_{pe}	q_{be}
WC	Ac	192.0 ±55.8	10.7 ± 2.05	65.5 ± 6.31
WC	Pr	6.12± 1.49	260.0 ±87.0	3.77± 2.58
WC	Bu	34.1 ± 0.26	5.95± 0.05	226.0 ±89.3
GC	Ac	195.0 ±65.5	19.0 ± 0.47	89.9 ±17.9
GC	Pr	38.8 ±12.8	422.0 ±19.5	8.29± 2.95
GC	Bu	63.4 ±12.8	9.63± 0.02	494.0 ±267.0

[a]Values are the mean steady-state total radioactivity ± standard error of the mean (n=2).

TABLE XLIII

^{14}C-TRANSFER RATIOS FOR RUMEN VFA'S DURING EACH INFUSION EXPERIMENT

Animal	Diet	Expt	^{14}C-Transfer Ratio x 100[a]			
			Y_{ai}	Y_{pi}	Y_{bi}	Y_{vi}
608	WC	Ac	-	7.31	45.9	36.0
608	WC	Pr	2.29	-	0.71	28.5
608	WC	Bu	10.0	3.03	-	-
422	WC	Ac	-	4.51	54.6	32.4
422	WC	Pr	2.52	-	2.92	26.4
422	WC	Bu	13.5	1.35	-	-
612	GC	Ac	-	4.55	71.9	36.1
612	GC	Pr	9.95	-	12.1	68.5
612	GC	Bu	9.84	0.94	-	-
608	GC	Ac	-	7.02	98.0	59.4
608	GC	Pr	20.9	-	4.71	60.4
608	GC	Bu	7.55	2.08	-	-

[a] ^{14}C-Transfer ratio defined as the specific activity of the specified VFA divided by the specific activity of the acid infused.

TABLE XLIV

MEAN ^{14}C-TRANSFER RATIOS FOR RUMEN VFA'S IN STEERS CONSUMING EITHER A WHOLE OR GROUND CORN RATION

Diet	Expt	^{14}C-Transfer Ratio x 100[a]			
		Y_{ai}	Y_{pi}	Y_{bi}	Y_{vi}
WC	Ac	-	5.91±1.4	50.3 ± 4.4	34.2 ± 1.8
WC	Pr	2.41± 0.12	-	1.82± 1.1	27.5 ± 1.1
WC	Bu	11.8 ± 1.8	2.19±0.84	-	-
GC	Ac	-	5.79±1.2	85.0 ±13.0	47.8 ±12.0
GC	Pr	15.4 ± 5.5	-	8.41± 3.7	64.5 ± 4.1
GC	Bu	8.70± 1.2	1.51±0.57	-	-

[a] ^{14}C-Transfer ratio defined as the specific activity of the specified VFA divided by the specific activity of the acid infused. Values are means ± standard error of the mean (n=2).

butyrate from extracellular acetate would involve the microbial uptake and metabolism of exogenous acetate. Bhat and Barker (1947); Davis (1967) and Satter and Esdale (1968) reported that lactate enhanced the incorporation of acetate into butyrate while diminishing the flow of butyrate carbon to acetate. Presumably acetate functions as an electron acceptor of the hydrogen formed during the conversion of lactate to pyruvate (Satter and Esdale, 1968). Although lactate levels were not measured in this study, Johnson et al. (1974) reported that feedlot steers consuming a GC ration had rumen lactate levels approximately five times higher than that observed in steers consuming a WC ration. In this study, steers fed the GC ration had a lower butyrate to acetate conversion than steers on the WC ration (9% vs 12%, respectively) which would agree with the rationale just presented.

Interconversions between propionate and the acetate and the butyrate compartments were much less, however there appeared to be a greater conversion of propionate carbon to acetate carbon with the GC ration as compared with the WC ration (15.0 vs 2.4%, respectively). The reason for this difference is not clear; however, predominant specie(s) of bacteria in the GC system may be metabolizing propionate via succinate to pyruvate which is then oxidized via the energy-yielding clastic reaction to acetate. This pathway could generate reducing equivalents from the succinate-fumarate couple and the conversion of malate to pyruvate by malic enzyme. This reaction sequence would quantitatively transfer the ^{14}C label in propionate to acetate since the propionate was labeled in the α position. Palmquist and Baldwin (1966) reported a three fold increase in the activity of malic dehydrogenase in the rumen of concentrate-fed

cattle when compared to cattle fed alfalfa hay.

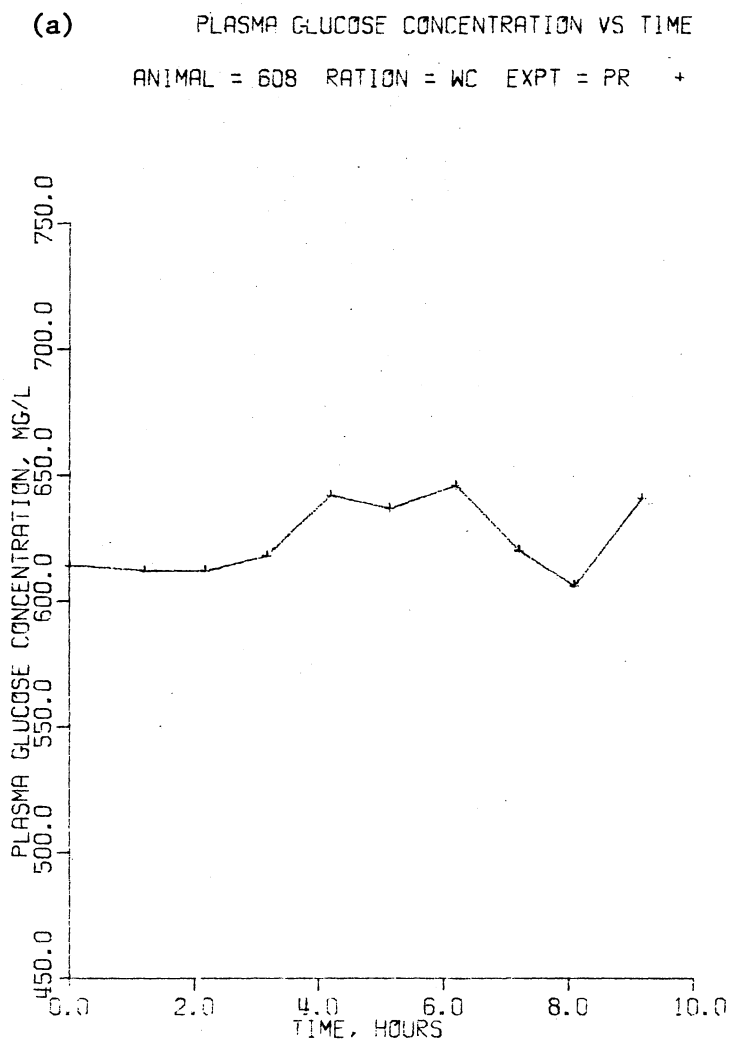
The conversion of acetate and propionate carbon to valerate was substantial on both rations, but conversion was greater with the GC ration. This observation parallels the previous discussion concerning the acetate to butyrate conversion. Similarly, the formation of valerate, presumably a condensation of acetate and propionate, may be acting as an electron sink in response to an elevated lactic acid production. The conversion of acetate to valerate during the 612-GC period was similar to values observed in steers consuming the whole corn diet; however, the remaining ^{14}C transfer rations describing the conversion of acetate and propionate carbon to valerate on the GC diet were nearly twice as high as the corresponding values observed on the whole corn diet. The reason for this apparent discrepancy in the 612-GC period is not clear.

Blood Parameters

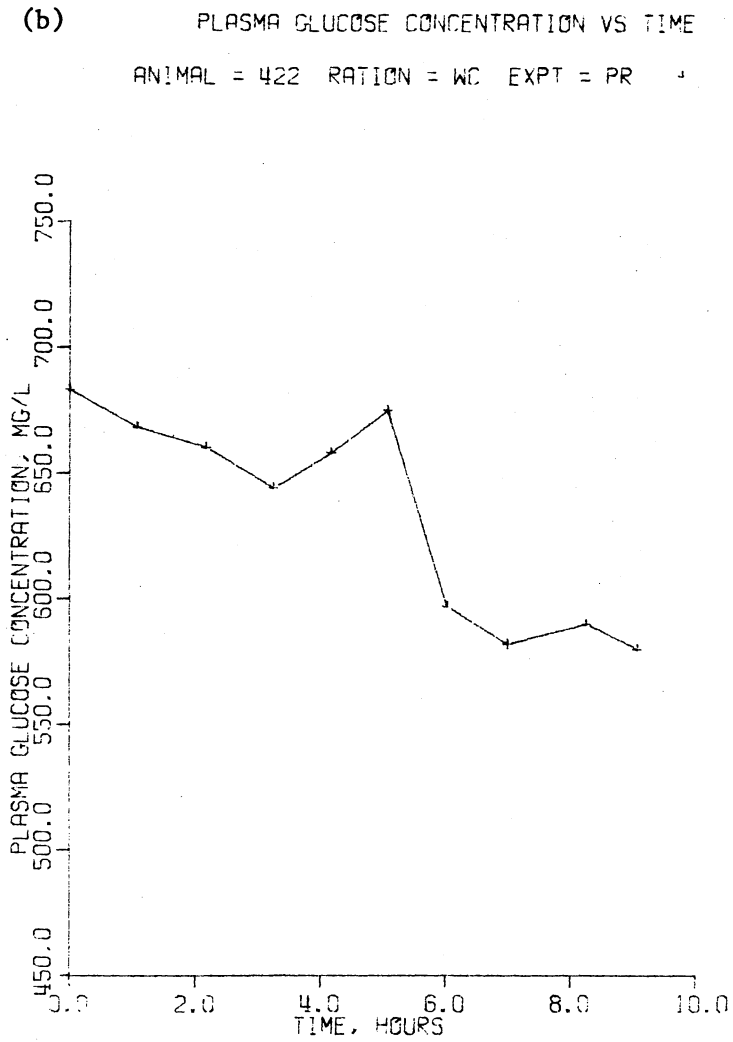
Plasma Glucose Concentration. The concentration of plasma glucose during each propionate infusion experiment is shown in Figure 48. The mean plasma glucose concentration for each propionate infusion and the average of the five and six hour samples for the remaining infusions are presented in Table XLV. The concentration of glucose over the infusion period was quite variable. The reasons for this are not clear; however, this may be related to the method of animal restraint and other stress situations during the infusion experiments. For example, a sharp rise in plasma glucose concentration occurred during the last part of the 612-GC-Pr infusion (Figure 48 c) immediately following the surgical reinstallation of the jugular catheter. This

Figure 48. Plasma Glucose Concentration During Each Propionate Infusion Experiment. (a) 608-WC, (b) 422-WC, (c) 612-GC, (d) 608-GC.

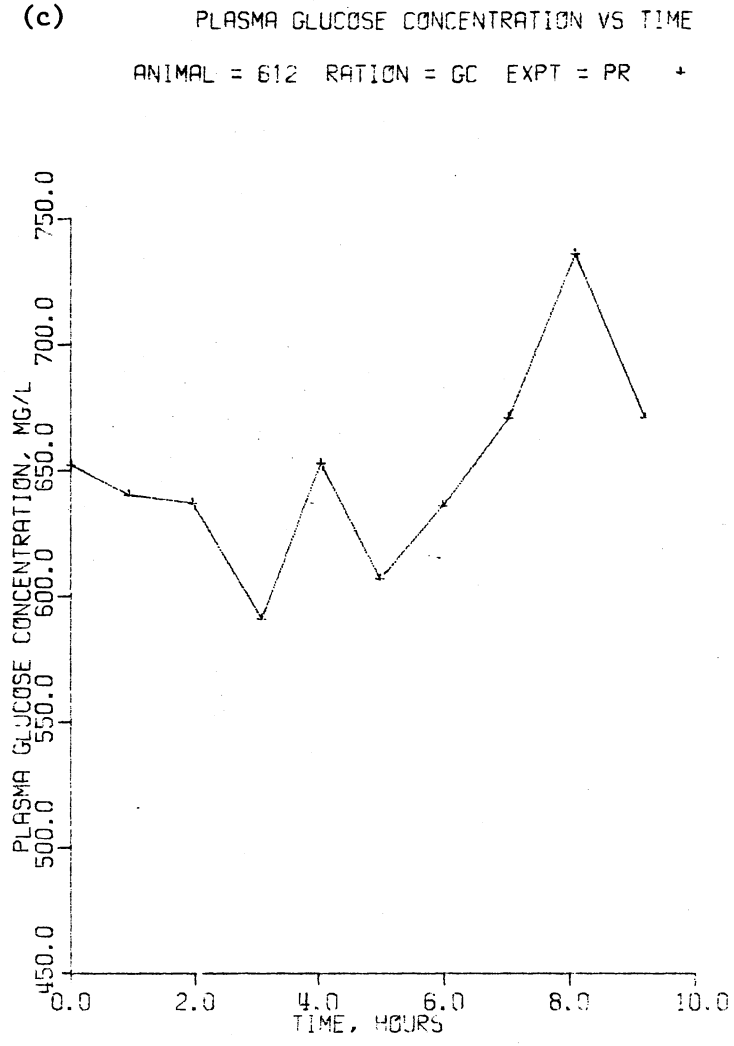
(a)


PLASMA GLUCOSE CONCENTRATION VS TIME
ANIMAL = 608 RATION = WC EXPT = PR +
PLASMA GLUCOSE CONCENTRATION, MG/L
450.0
500.0
550.0
600.0
650.0
700.0
750.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)


PLASMA GLUCOSE CONCENTRATION VS TIME
ANIMAL = 422 RATION = WC EXPT = PR +
PLASMA GLUCOSE CONCENTRATION, MG/L
450.0
500.0
550.0
600.0
650.0
700.0
750.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(c)



(d)

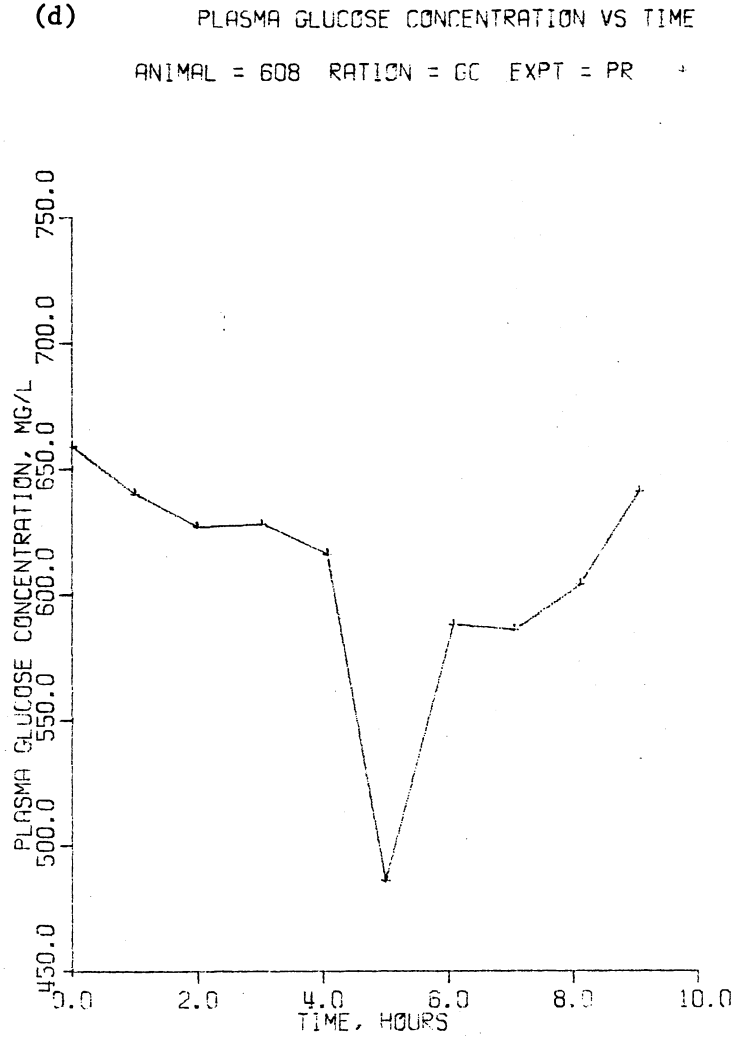


Figure 48. (Continued)

TABLE XLV

PLASMA GLUCOSE CONCENTRATION, STEADY-STATE SPECIFIC ACTIVITY AND Y_{gi} DURING EACH INFUSION EXPERIMENT[a]

Animal	Diet	Expt	[Glucose] (mg/l)	a_{ge}[b] (μCi/gac)	Y_{gi}[c] (x100)
608	WC	Ac	592± 0.7 (2)	2.82±0.12 (2)	7.52
608	WC	Pr	626±15.4 (10)	41.7 ±0.31 (3)	47.6
608	WC	Bu	634± 1.4 (2)	3.89±0.20 (2)	4.07
422	WC	Ac	670±17.7 (2)	3.79±0.17 (2)	8.77
422	WC	Pr	634±41.5 (10)	27.5 ±0.79 (3)	48.2
422	WC	Bu	657±55.9 (2)	4.37±0.12 (2)	6.41
612	GC	Ac	647±37.5 (2)	7.16±0.69 (2)	8.98
612	GC	Pr	649±39.6 (10)	27.3 ±0.25 (2)	66.3
612	GC	Bu	668±16.3 (2)	5.75±0.15 (2)	3.90
608	GC	Ac	738± 7.1 (2)	4.59±0.09 (2)	8.24
608	GC	Pr	621±24.8 (9)	24.4 ±0.81 (3)	43.5
608	GC	Bu	672±10.6 (2)	4.38±0.08 (2)	1.95

[a] Values shown are the infusion mean ± standard deviation. Numbers in parenthesis indicate the number of observations for each mean.

[b] Steady-state specific activity of plasma glucose adjusted to an infusion rate of 2.0 μCi/min.

[c] ^{14}C-Transfer ratio x 100 for the conversion of carbon in the i^{th} acid being infused to glucose carbon.

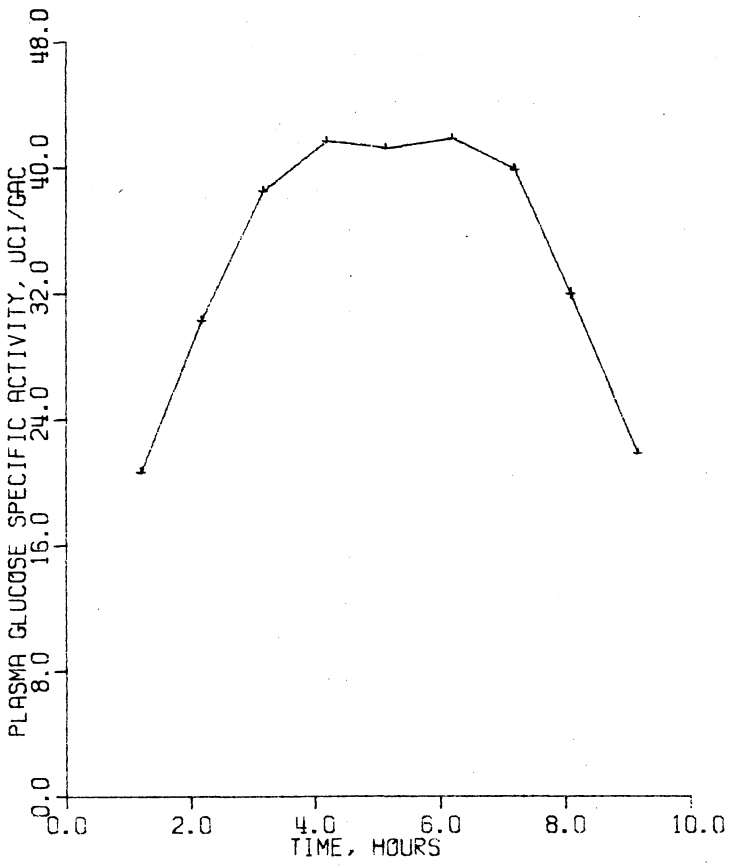
stress may have caused release of epinephrine which elicits mobilization of glucose from glycogen (Bassett, 1975).

Plasma Glucose Specific Activity. The plasma glucose specific activity during each propionate infusion experiment is illustrated in Figure 49. The mean steady-state plasma glucose specific activity and ^{14}C-transfer ratios for each infusion experiment are listed in Table XLV. The means for each ration are given in Table XLVI. Specific activity of the plasma glucose appeared to reach a steady-state at least two hours before the end of each propionate infusion. The steady-state plasma glucose specific activity obtained during the propionate infusions are a function of not only the specific activity of the ruminal propionate compartment and the rate of transfer of propionate carbon to glucose, but also the rates of carbon flow to glucose from amino acids, glycerol, lactate and exogenous glucose absorbed from the lower gut. Since the turnover (irreversible disposal) of the glucose carbon pool and the contribution of other metabolites to plasma glucose carbon were not determined, it is difficult to accurately assess the contribution of the rumen propionate carbon to glucose carbon.

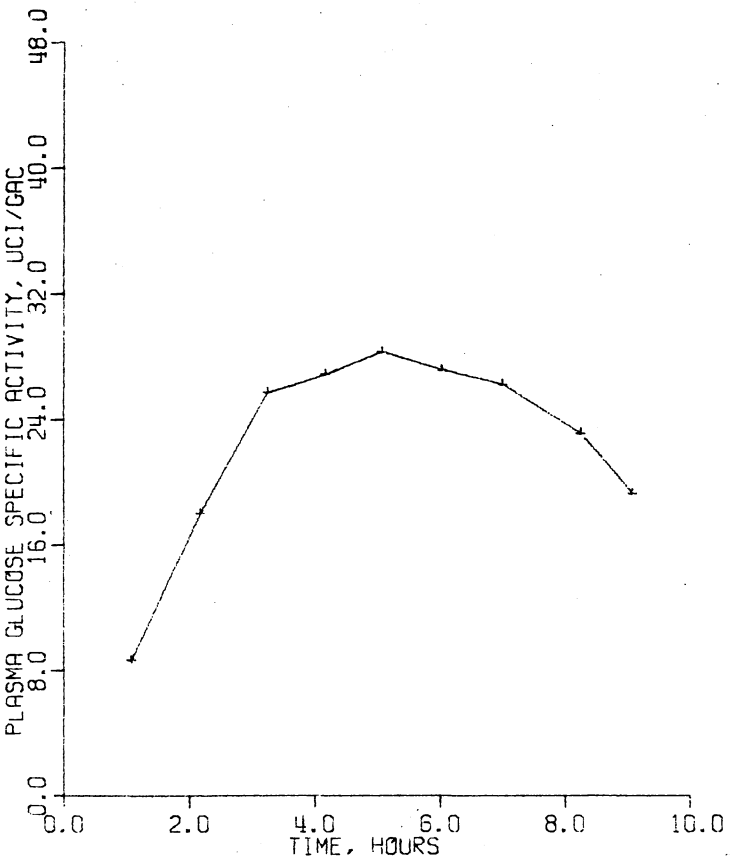
The apparent contribution of propionate to glucose can be estimated from the ^{14}C-transfer ratio of propionate carbon to glucose carbon; however, these values would underestimate the contribution of propionate to glucose if a fraction of the propionate radioactivity is lost via the citric acid cycle (Krebs and Lowenstein, 1960). In contrast, any recycling of ^{14}C back to glucose or to a glucose intermediate would cause the Y_{gi} values in Tables XLV-XLVI to overestimate

Figure 49. Plasma Glucose Specific Activity versus Time for Each Propionate Infusion Experiment. (a) 608-WC, (b) 422-WC, (c) 612-GC, (d) 608-GC.

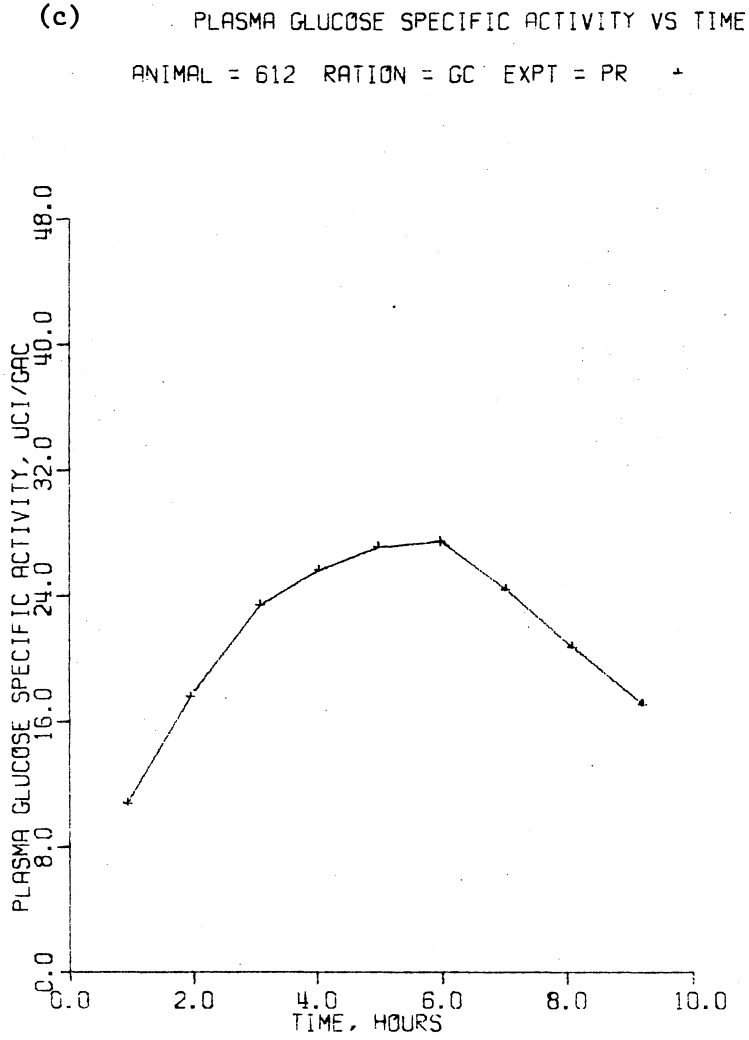
(a)


PLASMA GLUCOSE SPECIFIC ACTIVITY VS TIME
ANIMAL = 608 RATION = WC EXPT = PR +
PLASMA GLUCOSE SPECIFIC ACTIVITY, UCI/GAC
0.0
8.0
16.0
24.0
32.0
40.0
48.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(b)


PLASMA GLUCOSE SPECIFIC ACTIVITY VS TIME
ANIMAL = 422 RATION = WC EXPT = PR +
PLASMA GLUCOSE SPECIFIC ACTIVITY, UCI/GAC
0.0
8.0
16.0
24.0
32.0
40.0
48.0
0.0
2.0
4.0
6.0
8.0
10.0
TIME, HOURS

(c)



(d)

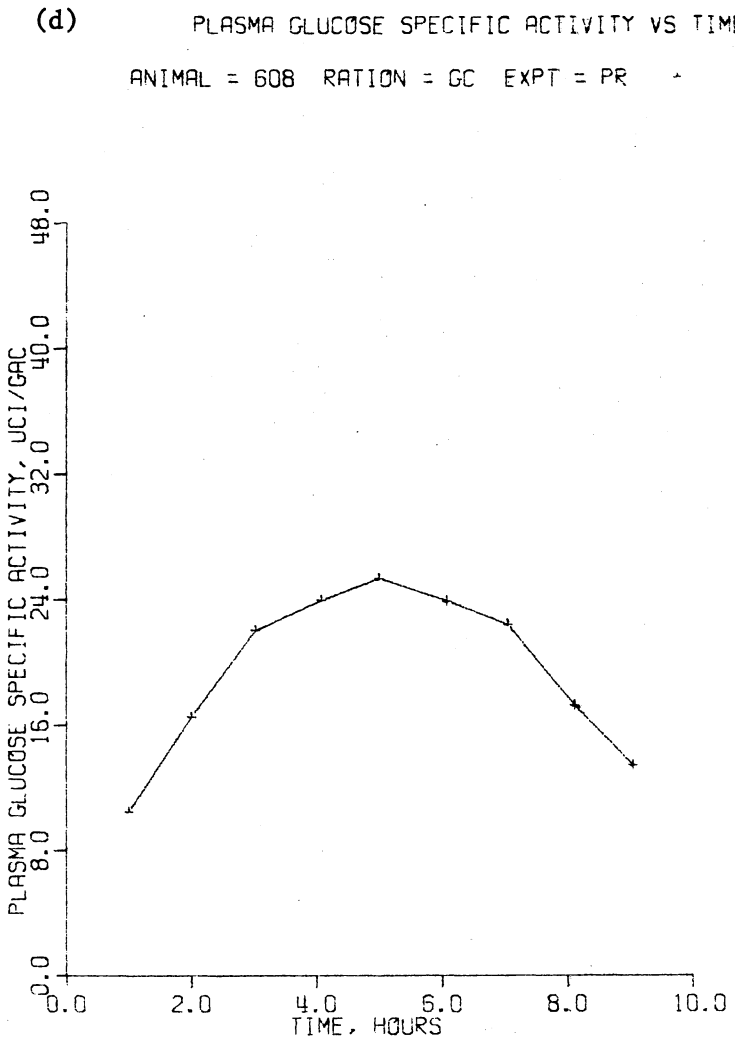


Figure 49. (Continued)

TABLE XLVI

MEAN PLASMA GLUCOSE CONCENTRATION AND Y_{gi} FOR STEERS CONSUMING EITHER A WHOLE OR A GROUND CORN DIET

Diet	[Glucose][a] (mg/l)	Transfer Rate x 100[b]		
		Y_{ga}	Y_{gp}	Y_{gb}
WC	636±11.0	8.15±0.63	47.9± 0.30	5.24±1.17
GC	666±16.2	8.61±0.37	54.9±11.4	2.93±0.98

[a]Mean ± standard error of the mean (n=6).

[b]^{14}C-Transfer ratio for the conversion of the specified VFA carbon to glucose carbon. Mean ± standard error of the mean (n=2).

the contribution of propionate carbon to glucose carbon. The relative magnitude of these errors were not determined in this study. Wiltrout and Satter (1972) estimated that 26% of the ^{14}C in propionate entering the citric acid cycle is lost due to the randomization (crossover) of carbon with other four carbon intermediates. Black et al. (1966) estimated that 11-14% of the acetyl-CoA entering the TCA cycle had been originally derived via the decarboxylation of oxaloacetate.

Incorporation of propionate carbon into glucose was similar for the periods 608-WC, 422-WC and 608-GC (47.6, 48.2 and 43.5%, respectively), but that observed during the 612-WC period was considerably higher (66.3%). This observation may be related to an increased rate of propionate production resulting in an increase in the proportion of glucose synthesized from propionate. Judson and Leng (1973b) reported an increase in the proportion of glucose synthesized from propionate when ruminal propionate production rate was increased. Values for the incorporation of propionate carbon into glucose carbon listed in Table XLVI are similar to those reported by other workers (Leng et al., 1967; Wiltrout and Satter, 1972).

The incorporation of acetate and butyrate carbon into glucose (Tables XLV and XLVI) probably reflects the randomization of carbon in the citric acid cycle, since de novo synthesis of glucose from either of these substrates has not been demonstrated. Generally, the extent of incorporation of acetate into glucose was greater than that observed with butyrate. This most likely is due to the greater metabolic activity of acetate relative to butyrate and possibly the conversion of most of the butyrate to β-hydroxybutyrate by the rumen epithelium

(Fell and Weeks, 1975). The values for Y_{ga} and Y_{gb} in Table XLVI are similar to those reported by Wiltrout and Satter (1972).

Compartmental Models of VFA Interconversion and Production

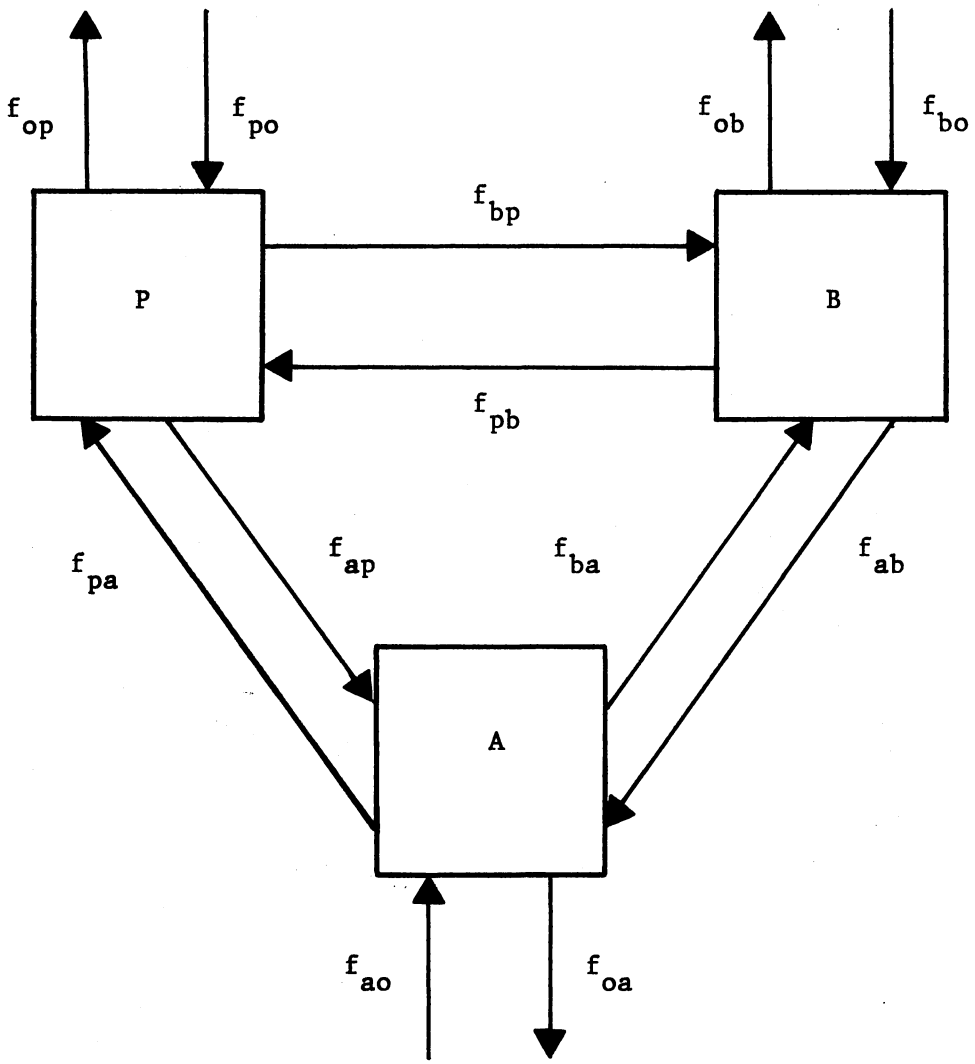
This section will primarily deal with the presentation and evaluation of various compartment models describing volatile fatty acid interconversion and production in the rumen of steers consuming either whole corn or ground high-concentrate rations. It should be emphasized that these models are not predictive models, but descriptive models that may be used as a tool to aid in the understanding and evaluation of ruminal VFA metabolism.

First, a complete three compartment model will be considered which, coupled with the error propagation algorithm developed in Chapter II, estimates the turnovers and turnover rate constants associated with the various compartments. Secondly, an incomplete three compartment system (Bergman et al., 1965) will be expanded to a four compartment model. Finally, the model will be evaluated on the basis of its ability to reconstruct the observed experimental data. This will be accomplished by computer simulation of the four compartment model using the derived model parameters and the initial conditions of each isotope infusion experiment.

Complete Three Compartment Model

The model to be considered is presented in Figure 50. It should be recognized that no experimental evidence exists to support the direct coupling of the propionate and butyrate compartments. Further-

Figure 50. The Complete Three Compartment VFA Model. A- acetate compartment, B- butyrate compartment, P- propionate compartment. Subscripts are a- acetate, b- butyrate, p- propionate, o- outside the system. The f_{ij} represents the flow of carbon (mgac/min) from compartment j to compartment i.


f_{op}
f_{po}
f_{ob}
f_{bo}
f_{bp}
P
B
f_{pb}
f_{pa}
f_{ap}
f_{ba}
f_{ab}
A
f_{ao}
f_{oa}

more, any direct conversion of butyrate to propionate, presumably via an oxidative decarboxylating mechanism, would result in the loss of the butyrate ^{14}C label since the butyrate was initially labeled in the C_1 position. Most likely any carbon transfer from butyrate to propionate or vice versa proceeds through the acetate compartment. However, a complete compartmental system must be assumed in order to use the error propagation algorithm outlined by Equations (2.47) through (2.57). Solving Equation (2.54) using the acetate, propionate and butyrate steady-state specific activity data for each animal-ration period (Table XXXIX) yields the model parameters listed in Table XLVII. The flows corresponding to compartment input, f_{io}, and compartment output, f_{oi}, were calculated according to the following equations:

$$f_{io} = f_{ii} - \sum_{i \neq j} f_{ij} \tag{5.7}$$

$$f_{oi} = f_{ii} - \sum_{i \neq j} f_{ji} \tag{5.8}$$

where i,j = a,p,b.

The discussion of the turnover rates observed with each animal and diet will be presented in the section dealing with the incomplete model, since the model parameters were only slightly altered by the exclusion of the f_{pb} and f_{bp} terms. Examination of the f_{pb} and f_{bp} terms listed in Table XLVII seems to support the initial assumption that these terms are zero or close to zero. For example, the data from 608-WC and 608-GC gave negative f_{bp} values which indicates that the model in Figure 50 does not accurately describe these data. Furthermore, the

TABLE XLVII

TURNOVER RATES AND THEIR ASSOCIATED ERROR TERMS FOR THE COMPLETE THREE COMPARTMENT VFA MODEL

Parameters[c] / Period[d]	Term[e]	faa	foa	fao	fap	fab	fpp	fop	fpo	fpa	fpb	fbb	fob	fbo	fba	fbp
608-WC	m	52.70	34.54	46.27	1.172	5.259	33.96	32.91	31.03	2.106	0.8202	34.88	28.80	18.95	16.05	-0.1209
	e	1.362	1.436	1.363	0.05148	0.03263	0.6225	0.6250	0.6260	0.04641	0.04315	1.134	1.135	1.221	0.4513	0.01727
422-WC	m	56.84	36.00	47.97	1.207	7.666	38.51	36.75	36.63	1.567	0.3097	35.40	27.42	15.58	19.27	0.5485
	e	1.366	1.442	1.392	0.07465	0.2579	0.9722	0.9752	0.9731	0.006195	0.04113	0.7527	0.7967	0.8831	0.4616	0.01600
612-GC	m	32.83	20.66	26.75	2.883	3.195	47.84	44.26	45.59	1.998	0.2533	14.18	10.73	3.314	10.17	0.6962
	e	0.9666	1.042	0.969	0.07296	0.01219	0.6456	0.6515	0.7053	0.2715	0.008320	0.3892	0.3895	0.4812	0.2789	0.04815
608-GC	m	62.33	48.65	45.06	12.84	4.430	42.52	31.52	39.52	2.284	0.7126	11.51	6.367	1.954	11.40	-1.844
	e	2.127	2.170	2.131	0.1068	0.08594	1.433	1.437	1.435	0.08173	0.01445	0.3163	0.3281	0.5290	0.4239	0.01470
608-GC[f]	m	64.05	46.75	44.78	13.11	6.164	42.61	31.83	39.62	2.873	0.1183	14.57	8.288	2.474	14.43	-2.334
	e	2.111	2.182	2.115	0.1175	0.05293	1.434	1.439	1.438	0.1032	0.03034	0.4071	0.4116	0.6792	0.5433	0.01970
WC	m	54.67	35.31	47.05	1.200	6.421	36.10	34.71	33.67	1.859	0.5712	35.11	28.12	17.33	17.59	0.1905
	e	0.6352	1.005	1.085	0.1478	0.8676	2.255	2.282	2.278	0.2717	0.1786	0.1598	0.9001	0.8102	0.7295	0.3143
GC	m	42.92	30.63	32.87	6.483	3.570	44.99	39.10	42.45	2.002	0.5348	12.67	8.565	2.972	10.29	-0.5918
	e	13.80	14.09	13.88	0.1953	1.501	2.772	2.780	2.908	0.8264	0.2943	1.533	2.166	3.145	2.745	0.0794

[a] Values were calculated by solving Equation (2.54) using the data in Tables XXXIX and XL.

[b] Values are expressed in units of mgac/min.

[c] f_{ij} represents the flow of carbon from compartment j to compartment i.

[d] Animal-ration period or diet

[e] m-turnover rate; e-error term.

[f] Specific activity data for infusion 608-GC-Bu was replaced by that obtained during the 612-GC-Bu infusion.

remaining values for f_{bp} and f_{pb} can be nearly accounted for by the conversions of butyrate $\longleftrightarrow$ acetate $\longleftrightarrow$ propionate. For example, consider the data for the 422-WC period. Assume that

$$Y_{bp} = \frac{f_{bp}}{f_{bb}} \tag{5.9}$$

and that

$$Y_{bp} = Y_{ba} \cdot Y_{ap} , \tag{5.10}$$

then the flow from P to B through A can be calculated from Equation (5.9) as

$$f_{bp} = Y_{bp} \cdot f_{bb} . \tag{5.11}$$

Substitution of values for Y_{ba} and Y_{ap} from Table XLIII into Equation (5.10) yields 0.0138, which is the transfer ratio for propionate carbon to butyrate carbon via the acetate compartment. This value is somewhat smaller than the one given in Table XLIII. From Equation (5.11), the flow of propionate carbon through acetate to butyrate is 0.4871 mgac/min. This value accounts for nearly 89% of f_{bp} shown in Table XLVII suggesting that f_{bp} in Figure 50 may be zero or nearly zero. Calculated in a similar fashion, the flow of carbon from butyrate to propionate via acetate accounts for approximately 76% of the f_{pb} listed in Table XLVII. Similar calculations performed on the other data sets in Table XLVII seem to generally support these conclusions.

The substitution of the 612-GC-Bu data for the 608-GC-Bu data did

not markedly change the model parameters. This is surprising in light of the drastically different VFA patterns present during these experiments (Tables XXX and XXXII).

The average turnover rates and their associated error terms for the WC and GC diets are also presented in Table XLVII. These values were obtained by solving Equation (2.54) using the values for acetate, propionate and butyrate specific activity listed in Table XL.

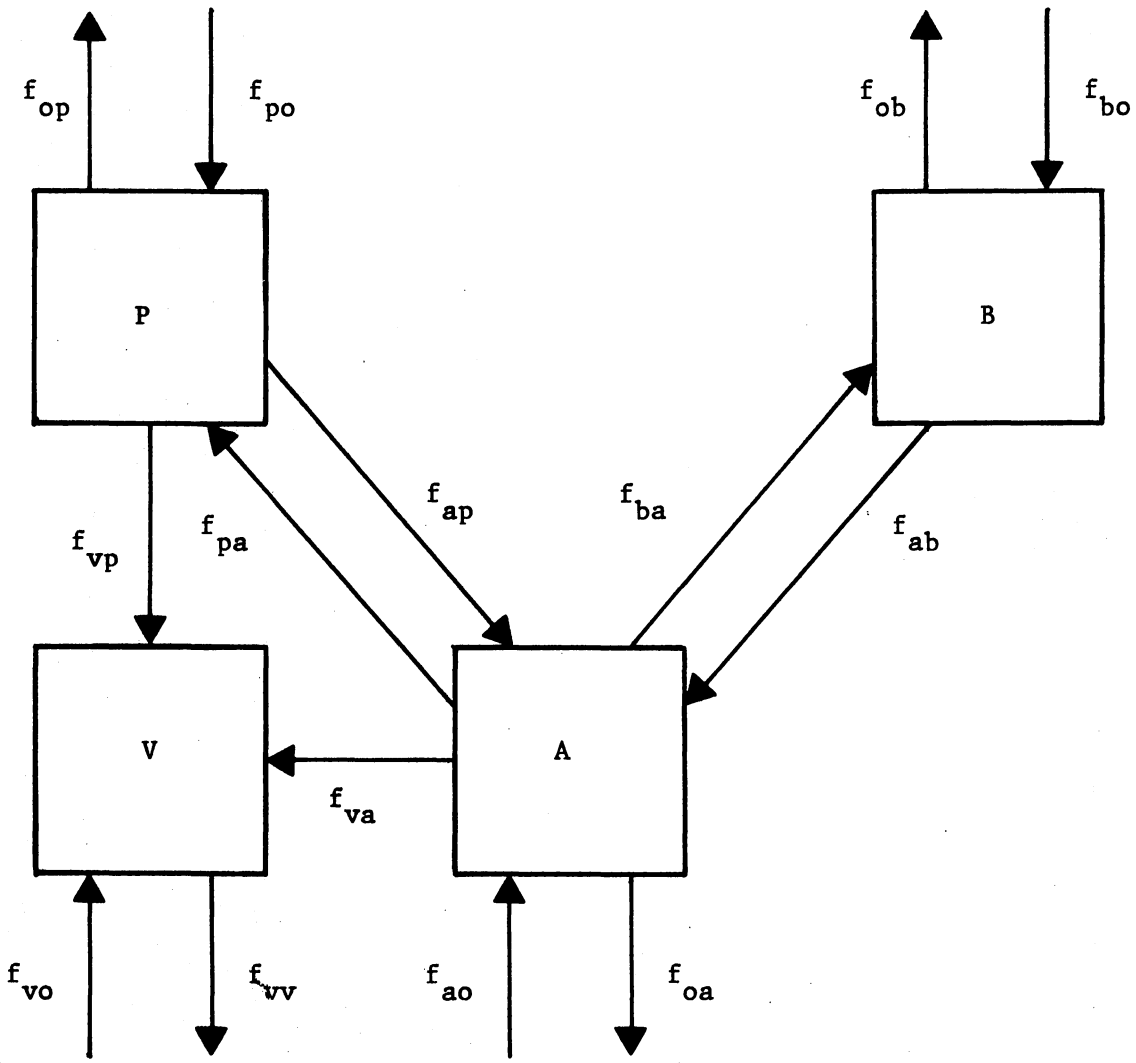
Estimates of the turnover rate constants (k_{ij}) associated with the flows indicated in Figure 50 were obtained by solving Equation (2.54) using the data listed in Table XLI and Table XLII. The turnover rate constants for each animal-ration period and each diet are tabulated in Table LIX, Appendix F.

Incomplete Four Compartment Model

The model to be considered is shown in Figure 51. In this model, f_{pb} and f_{bp} are assumed to be zero for reasons discussed in the previous section. Furthermore, f_{av} and f_{pv} were assumed to be zero because the design of the experiments did not permit the determination of these quantities. Although these flows are probably present in the real system, their contribution to the total turnover of the acetate and propionate compartments is probably quite small.

The calculation of the model parameters illustrated in Figure 51 was done in two steps. The acetate, propionate and butyrate compartments were treated as a separate system since the valerate compartment did not serve as an input to any of these compartments. Turnover rates for this sub-system for each animal-ration period and diet were calculated according to Equation (2.44) using the data listed in

Figure 51. The Incomplete Four Compartment VFA Model. A- acetate compartment, B- butyrate compartment, P- propionate compartment, V- valerate compartment. Subscripts are a- acetate, b- butyrate, p- propionate, v- valerate, o- outside the system. The flow of carbon (mgac/min) from compartment j to compartment i is represented by f_{ij}.


f_{op}
f_{po}
f_{ob}
f_{bo}
P
B
f_{vp}
f_{pa}
f_{ap}
f_{ba}
f_{ab}
V
A
f_{va}
f_{vo}
f_{vv}
f_{ao}
f_{oa}

Tables XXXIX and XL. When the system was overdetermined (more equations than unknowns), a least squares estimate was obtained as outlined by Equation (2.46). Turnover of the valerate compartment for each animal-ration period was estimated by the equation

$$f_{vv} = \tfrac{1}{2} \sum_{i=a,p} k_{vvi} Q_{vi} \qquad (5.12)$$

where Q_{vi} is the size of the valerate compartment during the i^{th} experiment and k_{vvi} is the turnover rate constant of the valerate pool during the i^{th} experiment. The k_{vvi} term was estimated from the slope of the ln a_{vi} versus time curve where $t > 6$ hours; i.e., after the infusion pump was turned off. This estimate, however, can at best be considered only a first approximation of k_{vv} since the 'washing out' of label in the valerate pool is confounded with label still entering from the acetate and propionate compartments. The net effect is to maintain a higher specific activity than that which would be observed during a similar period of specific activity decline due strictly to the entrance of unlabeled carbon. The turnover rates for each animal-ration period and each diet for the model in Figure 51 are listed in Table XLVIII.

Comparison of the f_{aa}, f_{pp}, f_{bb}, f_{ap}, f_{pa}, f_{ba}, f_{ab} terms in Table XLVIII to those in Table XLVII reveals that the elimination of the f_{pb} and f_{bp} terms has only a very minor influence on these model parameters. The remaining model parameters, namely f_{oi} and f_{io}, seem to be affected more heavily than the other model parameters; however, these effects are a combination to the elimination of f_{pb} and f_{bp} and

TABLE XLVIII

TURNOVER RATES FOR THE INCOMPLETE FOUR COMPARTMENT VFA MODEL FOR EACH ANIMAL-RATION PERIOD

Parameter[e] / Period[d]	faa	foa	fao	fap	fab	fpp	fop	fpo	fpa	fbb	fob	fbo	fba	fvv	fvo	fva	fvp
608-WC	52.70	33.42	46.27	1.172	5.259	33.95	32.31	31.29	2.656	34.88	29.62	18.85	16.03	1.651	0.5863	0.5942	0.4705
422-WC	56.84	35.06	47.97	1.207	7.666	38.50	36.77	36.67	1.831	35.40	27.73	16.09	19.31	1.982	0.8169	0.6420	0.5231
612-GC	32.83	19.47	26.75	2.883	3.195	47.82	43.36	45.52	2.296	14.18	10.99	3.95	10.23	2.300	-0.1063	0.8303	1.576
608-GC	62.33	46.49	45.06	12.84	4.430	42.74	28.77	38.56	4.179	11.47	7.040	0.920	10.55	1.874	-0.3710	1.113	1.132
608-GC[e]	64.05	46.51	44.78	13.11	6.164	42.67	28.43	39.51	3.159	14.44	8.276	1.170	13.27	1.874	-0.3710	1.113	1.132
WC	54.67	34.15	47.05	1.199	6.421	36.09	34.39	33.80	2.293	35.11	28.69	17.50	17.61	1.817	0.7021	0.6181	0.4968
GC	42.92	28.89	32.87	6.483	3.570	45.01	37.17	42.14	2.872	12.67	9.10	2.480	10.19	2.087	-0.2387	0.9717	1.354

[a]Values were calculated by solving Equations (2.44) and (2.46) using the data in Tables XXXIX and XL assuming fpb, fbp, fav, fpv, fbv, fvb = 0.

[b]Values are expressed in units of mgac/min.

[c]f_{ij} represents the flow of carbon from compartment j to compartment i.

[d]Animal-ration period or diet.

[e]Specific activity data for infusion 608-GC-Bu was replaced by that obtained during the 612-GC-Bu infusion.

the addition of the f_{va} and f_{vp} terms.

Animals 608 and 422 had similar acetate turnover rates when WC was consumed. Animal 608 had nearly twice the acetate turnover as did Animal 612 on the GC diet. Animal 608 had a higher (18%) acetate turnover when consuming GC as compared to that observed on WC. These observations parallel the differences seen in f_{oa}, the net acetate production rate. However, comparison of f_{ao}, the gross rate of acetate production, across animals and diets reveals that during periods 608-WC, 422-WC and 608-GC similar values for f_{ao} occurred, while during 612-GC nearly half as much carbon entered the extracellular acetate compartment as did during the other three periods. The incorporation of acetate carbon into propionate carbon was higher in Animal 608 when GC was consumed as compared to WC. Furthermore, f_{pa} appeared to be higher during 612-GC as compared to 422-WC.

The flow of carbon from acetate to butyrate, f_{ba}, was higher on WC than on GC. Although values for f_{ba} were similar for the animals on GC, Animal 422 had a higher f_{ba} than Animal 608 when WC was fed. However, the percentage of butyrate carbon turnover arising from acetate (Y_{ba}) is 46.0, 54.6, 72.1 and 92.0 for the periods 608-WC, 422-WC, 612-GC and 608-GC, respectively. These values agree very closely with those listed in Table XLIII. These data seem to suggest that on WC the majority of microbes producing acetate from feed substrates (presumably glucose polymers) subsequently convert a larger proportion of this acetate to butyrate intracellularly before it is excreted than do the majority of microbes producing acetate from GC substrates. Presumably under the conditions of GC feeding one or several species of bacteria are producing and excreting acetate while

a (several) different specie(s) of bacteria take up extracellular acetate and form butyrate. Satter and Esdale (1968) suggest, as discussed previously, that these latter species of bacteria belong to the class of lactate utilizers.

The turnover of propionate carbon appears to be higher on GC as compared to WC. However, when propionate input (f_{po}) and output (f_{op}) rates are considered, the periods 608-WC, 422-WC and 608-GC have similar values, but these are lower than that obtained during the 612-GC period. This increased net production rate of propionate during 612-GC may explain the increased transfer of propionate carbon to glucose carbon (Table XLV), in agreement with the studies of Judson and Leng (1973b). The conversion of propionate to acetate was lower on WC than on GC. An exceptionally high flow of carbon from propionate to acetate occurred during the 608-GC period. A plausible explanation for this observation was presented earlier in this chapter.

The turnover of the butyrate compartment was markedly greater on WC than on GC (more than doubled). Examination of the extracellular butyrate compartment input (f_{bo}) and output (f_{ob}) indicates that considerably more butyrate was produced on the WC diet. Furthermore, the conversion of butyrate carbon to acetate carbon was greater on WC as compared to GC. This was also true when f_{ab} was expressed relative to f_{aa}. These observations tend to support the lactate utilization theory of Satter and Esdale (1968).

The turnover of valerate appeared to be similar across all periods; however, f_{vv} was slightly lower during the 608-WC period. Again, it should be emphasized that these estimates are probably minimum values since entry of label from acetate or propionate will,

in effect, simulate a recycling of label back to valerate resulting in an underestimation of the true valerate turnover. The flow of carbon from the acetate or the propionate pool to the valerate pool was calculated from the estimate of f_{vv} and Y_{vi} (Table XLIII) such that

$$f_{vi} = f_{vv} \cdot Y_{vi} . \tag{5.13}$$

There appeared to be a tendency for f_{va} and f_{vp} to be higher on GC as compared to WC. However, this trend may be a reflection of the tendency for Y_{vi} to be higher on GC (Table XLIII).

Estimates of the turnover rate constants (k_{ij}) associated with the flows in Figure 51 were obtained by solving Equations (2.44) and (2.46) using the data listed in Tablex XLI and XLII. These turnover rate constants are tabulated in Table LX, Appendix F.

VFA Production and Its Relationship to the Amount of Substrate Fermented

The numbers of moles of hexose degraded to VFA's can be estimated from Equations (3.24) - (3.27) such that

$$f_h = \frac{f_{oa}}{4} + \frac{f_{op}}{6} + \frac{f_{ob}}{4} + \frac{f_{vv}}{5} \tag{5.14}$$

where f_h is the number of moles of hexose metabolized per unit time. Table XLIX lists the calculated values for f_h for each animal-ration period and for each ration. These data suggest that a higher proportion of the dietary carbohydrate (starch) is fermented to VFA's on the WC ration.

Cole (1975) found values for the starch content of whole corn to

TABLE XLIX

EXTENT OF RUMEN CARBOHYDRATE DIGESTION BASED ON THE RATES OF VFA PRODUCTION

Period	Quantity Fermented/hr		Starch[c] Intake g/hr	%[d] Ruminal Digestion
	moles hexose[a]	g starch[b]		
608-WC	1.289	209	202	104
422-WC	1.333	216	202	107
612-GC	0.9181	149	202	73.8
608-GC	1.113	180	202	89.1
WC	1.308	212	202	105
GC	0.9666	157	202	77.7

[a]Calculated by Equation (5.15) from data in Tables LI and LII.

[b]Assumes that hexose in starch has a molecular weight of 162 g/mole.

[c]Assumes that the corn was 80% starch on a dry matter basis; 0.80 x 0.84 x 300 g/hr = 202 g/hr.

[d]b/c x 100.

range from 72-80% on a DM basis. Hourly starch intakes are presented in Table XLIX assuming that the corn in this study contained 80% starch (DM basis) and that all the dietary starch came from corn. Steers consuming the WC ration appeared to digest all the starch entering the rumen while those consuming GC appeared to digest only 78% of the dietary starch. Cole et al. (1976a) reported ruminal starch digestibilities (as % of intake) in steers consuming 90 and 82% WC rations of 80 and 68%, respectively. McCullough and Matsushima (1973) reported ruminal starch digestibilities of 61% on WC. Waldo (1973), summarizing several studies where GC was fed to steers, reported that ground corn starch was about 72% digestible in the rumen.

The reason for the apparently higher ruminal starch digestion on WC as compared to GC is not clear. Perhaps rumination on the whole corn ration 'processed' the grain such that the starch in the kernel was hydrated more rapidly, thus making the starch granules more easily attacked by the microbes. This possibility, coupled with an increased retention of the larger feed particles of the whole corn diet as compared to the much smaller feed particles of the ground corn ration (see Table XII), could create conditions for a more complete ruminal starch digestion on the whole corn ration.

The values for VFA production listed in Table XLVIII probably represent an overestimation of the true production rates due to (1) problems discussed earlier concerning the attainment of a uniform steady-state in the rumen and obtaining representative rumen samples and/or (2) improper choice of a model describing VFA production and interconversion. Presumably VFA's, particularly acetate, are incorporated into cell components not accommodated in the model given in

Figure 51. For example, acetate and propionate carbon were incorporated into the 'isovaleric' fraction (see Tables LV-LVIII, Appendix E). Since production rates are essentially calculated by difference, any turnovers not accounted for by the compartmental interconnections would be included in the f_{oi} terms. However, it is difficult to conceive of conditions which would amplify these errors during the feeding of whole corn relative to that during the feeding of ground corn.

Sutton (1972) has emphasized the importance of obtaining representative samples of rumen contents when studying VFA production rates in bovines. If the ruminal distribution of whole corn digestion is different from that occurring with ground corn, then site of sampling may be a very important determinant influencing the conclusions as to the relative rates of VFA production on these two rations. Sampling a site in the rumen where whole corn digestion is more localized as compared to that for ground corn could possibly explain the conclusions obtained from this experiment. Since samples were taken from the lower portion of the ventral sac, this hypothesis seems tenable.

Model Simulation

The model in Figure 51 can be expressed as a system of differential equations describing the quantity of tracer in each compartment as a function of time. This system,

$$\dot{q}_a(t) = u_a(t) + f_{ap}\, a_p(t) + f_{ab}\, a_b(t) - f_{aa}\, a_a(t) \qquad (5.15)$$

$$\dot{q}_p(t) = u_p(t) + f_{pa}\, a_a(t) - f_{pp}\, a_p(t) \qquad (5.16)$$

$$\dot{q}_b(t) = u_b(t) + f_{ba}\, a_a(t) - f_{bb}\, a_b(t) \qquad (5.17)$$

$$\dot{q}_v(t) = f_{va}\, a_a(t) + f_{vp}\, a_p(t) - f_{vv}\, a_v(t)\ , \qquad (5.18)$$

was simulated for each infusion experiment on an IBM 370/158 computer using the CSMP similation language given the initial compartmental conditions at t=0 and the turnover rates from Table XLVIII. The simulation program is listed in Table L.

The steady-state specific activity of each compartment during each simulated infusion experiment was calculated from the average of the specific activity observed at five and six hours after the start of the 'experiment'. Table LI compares the simulated steady-state specific activities to the observed steady-state specific activities from Table XXXIX.

In general, the model appears to reconstruct the experimental data fairly accurately; however, several discrepancies are apparent. The prediction of specific activities a_{bp} and a_{pb} is quite poor. This seems to indicate that the models description of carbon transfer between the propionate and butyrate compartments is not accurately defined. The acetate and butyrate interconversions were simulated quite well for the whole corn diet, but are not well described for the ground corn diet. Reconstruction of the valerate data was usually acceptable considering the approximation method used to determine the turnover rates associated with this compartment. Substitution of the 612-GC-Bu data for the 608-GC-Bu data appeared to improve the reconstruction of some of the experimental observations, while sacrificing the accuracy of others.

TABLE L

FOUR COMPARTMENT VFA MODEL: CSMP VERSION

```
****CONTINUOUS SYSTEM MODELING PROGRAM****

*** VERSION 1.3 ***

TITLE WHOLE CORN-VFA RATE STUDY SIMULATION I 1-C(14)-ACETATE
TITLE ANIMAL 608
TITLE PRIMED DOSE, CONSTANT INFUSION WITH CUTOFF AT EQUILIBRIUM

* UNITS:  Q(X)  = UC  //TOTAL AMOUNT OF RADIOACTIVITY IN POOL X //
*         Q(XX) = MGAC //TOTAL AMOUNT OF UNLABELED SPECIES IN POOL XX//
*         F(XY) = MGAC/MIN //FLOW OF MATERIAL FROM POOL Y TO POOL X //
*         R(X)  = UC/MIN  // RATE OF INFUSION INTO POOL X //
*         S(X)  = UC/MGAC  // SPECIFIC ACTIVITY OF POOL X //
*         Q(X)O = UC //INITIAL CONDITION OF POOL X; PRIMING DOSE//

INITIAL

INCON QAO=241.1, QPO=0.0, QBO=0.0

PARAMETER QAC=4640.0, QPR=2380.0, QBU=2240.0, QVA=460.0

PARAMETER FAA=52.70, FAP=1.172, FAB=5.259

PARAMETER FPP=33.95, FPA=2.656

PARAMETER FBB=34.88, FBA=16.03

PARAMETER FVV=1.651, FVA=0.5942, FVP=0.4705

PARAMETER RA=2.0, RP=0.0, RB=0.0

PARAMETER XOFF=365.0

      QVO=0.0
      QA=QAO
      QP=QPO
      QB=QPO
      QV=QVO

DYNAMIC

NOSORT

      Y = 1 - STEP(XOFF)
      X1 = Y * RA
      X2 = Y * RP
      X3 = Y * RB
      DQADT = X1 - FAA*SA + FAP*SP + FAB*SB
      DQPDT = X2 + FPA*SA - FPP*SP
      DQBDT = X3 + FBA*SA         - FBB*SB
      DQVDT =      FVA*SA + FVP*SP - FVV*SV
```

TABLE L (Continued)

```
      QA = INTGRL( QAC, DQADT )
      QP = INTGRL( QPO, DQPDT )
      QB = INTGRL( QBO, DQBDT )
      QV = INTGRL( QVO, DQVDT )
      SA = QA / QAC
      SP = QP / QPR
      SB = QB / QBU
      SV = QV / QVA
TIMER FINTIM=720.0, DELT=5.0, OUTDEL=5.0

PRTPLOT SA( QA, DQADT )
LABEL SPECIFIC ACTIVITY, ACETATE (UC/MGAC ADJ TO SIR, SSW, SFR)

PRTPLOT SP( QP, DQPDT )
LABEL SPECIFIC ACTIVITY, PROPIONATE (UC/MGAC ADJ TO SIR, SSW, SFR)

PRTPLOT SB( QB, DQBDT )
LABEL SPECIFIC ACTIVITY, BUTYRATE (UC/MGAC ADJ TO SIR, SSW, SFR)

PRTPLOT SV( QV, DQVDT )
LABEL SPECIFIC ACTIVITY, VALERATE (UC/MGAC ADJ TO SIR, SSW, SFR)


END


TITLE WHOLE CORN-VFA RATE STUDY SIMULATION II 2-C(14)-PROPIONATE

TITLE ANIMAL 608

TITLE PRIMED DOSE, CONSTANT INFUSION WITH CUTOFF AT EQUILIBRIUM

INCON QAC = 0.0, QPO = 183.4
PARAMETER RA = 0.0, RP = 2.0
PARAMETER XOFF = 360.0

END


TITLE WHOLE CORN-VFA RATE STUDY SIMULATION III 1-C(14)-BUTYRATE

TITLE ANIMAL 608

TITLE PRIMED DOSE, CONSTANT INFUSION WITH CUTOFF AT EQUILIBRIUM

INCON QPC = 0.0, QBO = 240.9
PARAMETER RP = 0.0, RB = 2.0
PARAMETER XOFF = 365.0

END
STOP


ENDJOB
```

TABLE LI

COMPARISON BETWEEN OBSERVED AND SIMULATED STEADY-STATE SPECIFIC ACTIVITIES FOR EACH INFUSION EXPERIMENT

Period	Expt	a_{ae}			a_{pe}			a_{be}			a_{ve}		
		Obs[a]	Sim[b]	%Δ[c]	Obs	Sim	%Δ	Obs	Sim	%Δ	Obs	Sim	%Δ
608-WC	Ac	39.8	40.1	0.7	2.91	3.16	7.9	18.3	18.5	1.1	14.4	11.0	30.9
608-WC	Pr	1.35	1.35	0.0	59.0	59.2	0.3	0.42	0.60	30.0	16.8	12.5	34.4
608-WC	Bu	6.04	6.08	0.7	1.83	0.47	289.0	60.1	60.4	0.5	-	1.46	-
422-WC	Ac	38.0	38.0	0.0	1.71	1.65	3.6	20.7	20.5	1.0	12.4	7.95	56.0
422-WC	Pr	1.31	1.06	23.6	52.0	48.9	6.3	1.52	0.48	217.0	13.7	6.77	102.0
422-WC	Bu	8.24	8.10	1.7	0.83	0.31	168.0	61.0	60.9	0.2	-	1.37	-
612-GC	Ac	65.8	63.2	4.1	3.00	2.57	16.7	47.4	40.2	17.9	23.8	18.1	31.5
612-GC	Pr	4.18	3.11	34.4	42.0	40.0	5.0	5.06	1.62	212.0	28.7	20.5	40.0
612-GC	Bu	14.9	13.2	12.9	1.43	0.46	211.0	151.8	149.3	1.7	-	3.14	-
608-GC	Ac	35.0	34.7	0.9	2.46	3.31	25.7	34.3	27.6	24.3	20.8	18.1	14.9
608-GC	Pr	9.96	9.90	0.6	47.6	47.1	1.1	2.25	6.36	64.6	28.8	24.5	17.6
608-GC	Bu	14.1	11.7	20.5	3.89	0.94	314.0	187.1	169.1	10.6	-	4.52	-
608-GC	Ac	35.0	34.7	0.9	2.46	2.41	2.1	34.3	31.1	10.3	20.8	18.2	14.3
608-GC	Pr	9.96	9.69	2.8	47.6	45.5	4.6	2.25	7.36	69.4	28.8	23.3	23.6
612-GC	Bu	14.9	14.6	2.1	1.43	0.88	62.5	151.8	150.7	0.7	-	6.29	-

[a] Observed steady-state specific activity (Table XXXIX).

[b] Simulated steady-state specific activity.

[c] $\frac{|\text{Obs} - \text{Sim}|}{\text{Sim}} \times 100 = \% \Delta$

CHAPTER VI

SUMMARY

Large numbers of beef cattle in the United States are fed diets containing largely concentrates. These concentrates contain primarily starch, which is either fermented in the rumen to volatile fatty acids, CO_2, methane and microbial cells or, by-passed out of the rumen to the small intestine where enzymatic digestion produces glucose which is then absorbed and metabolized by the animal. The relative partition between these two major routes of starch digestion may influence (1) the energetic efficiency of transforming feed energy into animal tissues and (2) the relative output of microbial protein due to complex interrelationships between ruminal nitrogen and carbohydrate metabolism. The production of volatile fatty acids is an important determinant in the overall efficiency of ruminant starch digestion. The relative and absolute rates of VFA production seem to be a logical means of evaluating the several methods of grain processing in use today. However, studies undertaking this task have been non-existent. It was hoped that this study would be a preliminary step toward this goal.

This study was conducted to evaluate the production and interconversion of VFA's in the rumen of beef steers consuming whole or ground corn high-concentrate diets. Steers were fed hourly in an attempt to create a steady-state rumen fermentation. Primed dose, constant infusion experiments were performed using ^{14}C-labeled acetate,

propionate or butyrate. A single dose of PEG was used as a non-absorbable marker to determine rumen liquid volume and rumen liquid outflow rates.

Rumen liquid volumes appeared to be relatively constant regardless of the physical form of the corn. However, rumen dilution rate tended to be higher in steers consuming the whole corn diet as compared to that observed in steers consuming the ground corn diet (11 vs 7.4%/hr, respectively).

There appeared to be a positive relationship between rumen liquid dilution rate and the ratio of 2-methylbutyric acid to 3-methylbutyric acid. This suggests that these branched-chain VFA's may play an important role in rumen bacteria growth rate.

Extracellular acetate pool size appeared to be similar on both diets. There appeared to be a higher propionate pool size on the ground corn diet, while the butyrate pool size tended to be higher on the whole corn diet.

VFA specific activity appeared to approach steady-state conditions throughout this study. In order to make valid comparisons across experiments and diets, these data were adjusted to an infusion rate of 2.0 μCi/min, standard steer weight of 400 kg and standard feed rate of 300 g/hr. This data was then used to determine the turnover rates associated with (1) a complete three compartment model (acetate, propionate, butyrate) with error propagation and (2) an incomplete four compartment model (acetate, propionate, butyrate, valerate).

The three compartment model was determined to inaccurately describe the VFA system due to biochemical considerations and the fact that the model gave a negative turnover rate (f_{bp}) for some of

the data sets. However, the agreement between the parameters of the three compartment model and those calculated for the four compartment model was quite good.

Net VFA production rates for acetate (f_{oa}) and butyrate (f_{ob}) were higher on the whole corn ration, while propionate (f_{op}) production was slightly higher on the ground corn diet. Rates of valerate production were similar across diet; however, the method by which these rates were determined probably resulted in their underestimation. Relative conversions of acetate to butyrate appeared to be higher while the conversion of butyrate to acetate tended to be lower on the ground corn diet as compared to the whole corn diet. In addition, the conversion of propionate to acetate was considerably higher on the ground corn diet as compared to the whole corn ration.

The relative contribution of propionate carbon to glucose carbon did not appear to vary across diet; however, during one ground corn experiment this value approached 66% as compared to 46% obtained in the other experiments. This apparent increase in the contribution of propionate to glucose production appeared to be related to an increase in ruminal propionate production during this particular experiment.

Results of this study suggest that the starch in whole corn is completely degraded to VFA in the rumen, while the starch in ground corn is only 80% degraded in the rumen. The reasons for this contridiction of current concepts concerning the ruminal by-pass of whole corn starch are not clear, but they may be related to differences in rumination, hydration of the starch granule and the relative retention times of feed particles occurring in these two diets.

Recommendations for Further Investigation

Future studies investigating the effects of grain processing on ruminal VFA production should consider the following suggestions in attempting to maximize the return of information from these experiments.

1. Due to the physical environment in the rumen, the primed dose, constant infusion method of tracer administration should continue to be the method of choice. Infusions lasting 2-3 hours longer than those used in this study may be valuable in increasing the precision of determining steady-state specific activity. The use of multiple level infusions (as discussed in Chapter II) to increase data precision should also be considered.
2. Collection of samples after the termination of the infusion and a subsequent transient analysis of the data (Gowdy and Mulholland, 1977) may provide additional system information.
3. Incorporation of the branched-chain and longer chain VFA's into the VFA model should be investigated. Furthermore, the consideration of VFA incorporation into microbial cell components should also be included.
4. Simultaneous determination of the relative sites and extents of starch digestion (Cole et al., 1976a) should be employed.
5. Simultaneous determinations of plasma glucose kinetics and the relative contributions of propionate and exogenous glucose to plasma glucose turnover would be desirable.
6. The significance and relative importance of fermentation in the large intestine should be investigated.

LITERATURE CITED

Adeeb, N. N., G. F. Wilson, and R. C. Campling. 1971. Physical form of maize and its digestibility in cattle and sheep. Anim. Prod. 13:391.

Allen, D. J., H. J. Reimers, I. A. Feuerstein, and J. F. Mustard. 1975. The use and analysis of multiple responses in multi-compartment cellular systems. Biometrics 31:921.

Allison, M. J. 1969. Biosynthesis of amino acids by ruminal micro-organisms. J. Anim. Sci. 29:797.

Allison, M. J. and M. P. Bryant. 1963. Biosynthesis of branched-chain amino acids from branched-chain fatty acids by rumen bacteria. Arch. Biochem. Biophys. 101:269.

Allison, M. J., J. A. Bucklin, and I. M. Robinson. 1966. Importance of the isovalerate carboxylation pathway of leucine biosynthesis in the rumen. Appl. Microbiol. 14:807.

Anderson, R. L. and W. A. Wood. 1969. Carbohydrate metabolism in microorganisms. Ann. Rev. Micro. 23:539.

Annison, E. F. 1954. Some observations on volatile fatty acids in the sheep's rumen. Biochem. J. 57:400.

Annison, E. F., R. A. Leng, D. B. Lindsay, and R. R. White. 1963. The metabolism of acetic acid, propionic acid and butyric acid in sheep. Biochem. J. 88:248.

Annison, E. F., D. B. Lindsay, and R. R. White. 1963a. Metabolic interrelations of glucose and lactate in sheep. Biochem. J. 88:243.

Annison, E. F. and R. J. Pennington. 1954. The metabolism of short-chain fatty acids in the sheep. 3. Formic, n-valeric and some branched-chain acids. Biochem. J. 57:685.

A.O.A.C. 1965. Official Methods of Analysis. Association of Official Agricultural Chemists, Washington, D.C., 10th edition.

Armstrong, D. G. and K. L. Blaxter. 1957a. The heat increments of steam volatile fatty acids in fasting sheep. Br. J. Nutr. 11:247.

Armstrong, D. G. and K. L. Blaxter. 1957b. The utilization of acetic, propionic and butyric acids by fattening sheep. Br. J. Nutr. 11:413.

Armstrong, D. G., K. L. Blaxter, and N. McC. Graham. 1957. The heat increments of mixtures of steam volatile fatty acids in sheep. Br. J. Nutr. 11:392.

Armstrong, D. G., K. L. Blaxter, N. McC. Graham, and F. W. Winman. 1958. The utilization of the energy of two mixtures of steam volatile fatty acids by fattening sheep. Br. J. Nutr. 12:177.

Artom, C., G. Sarzana, and G. Segres. 1938. Influence des grasses alimentaires sur la formation des phospholipides dons les tissues animaux (nouvelles recherches). Arch. Internat. Physiol. 47:245.

Ash, R. and G. D. Baird. 1973. Activation of volatile fatty acids in bovine liver and rumen epithelium. Evidence for control by auto-regulation. Biochem. J. 136:311.

Atwal, A. S. and F. D. Sauer. 1974. Enzymes of pyruvate and phospho-enolpyruvate carboxylation in rumen bacteria. Can. J. Anim. Sci. 54:595.

Ayers, W. A. 1959. Phosphorolysis and synthesis of cellobiose by cell extracts from Ruminococcus flavefaciens. J. Biol. Chem. 234:2819.

Bachofen, R., B. B. Buchanan, and D. I. Arnon. 1964. Ferredoxin as a reductant in pyruvate synthesis by a bacterial extract. Proc. Natl. Acad. Sci. U.S.A. 51:690.

Bailey, C. B. and C. C. Balch. 1961. Saliva secretion and its relation to feeding in cattle. 1. The composition and rate of secretion of parotid saliva in a small steer. Br. J. Nutr. 15:371.

Bailey, R. C., G. S. Eadie, and F. H. Schmidt. 1974. Estimation procedures for consecutive first order irreversible reactions. Biometrics 30:67.

Balch, C. C. 1958. Observations on the act of eating in cattle. Br. J. Nutr. 12:330.

Balch, D. A. and S. J. Rowland. 1957. Volatile fatty acids and lactic acid in the rumen of dairy cows receiving a variety of diets. Br. J. Nutr. 11:288.

Baldwin, R. L. 1965. Pathways of carbohydrate metabolism in the rumen in Physiology of Digestion in the Ruminant. R. W. Dougherty (ed.), Butterworths, Washington, D.C.

Baldwin, R. L. 1970. Metabolic functions affecting the contribution of adipose tissue to total energy expenditure. Fed. Proc. 29:1277.

Baldwin, R. L., L. J. Koong, and M. J. Ulyatt. 1977. The formation and utilization of fermentation end products: mathematical models in Microbiology of the Normal Gut. R. T. J. Clarke and T. Bauchop (eds.), Academic Press, Inc., New York. (In press).

Baldwin, R. L. and L. P. Milligan. 1964. Electron transport in Peptostreptococcus elsdenii. Biochem. Biophys. Acta 92:421.

Baldwin, R. L., W. A. Wood, and R. S. Emery. 1962. Conversion of lactate-^{14}C to propionate by the rumen microflora. J. Bacteriol. 83:907.

Baldwin, R. L., W. A. Wood, and R. S. Emery. 1963. Conversion of glucose-^{14}C to propionate by the rumen microbiota. J. Bacteriol. 85:1346.

Baldwin, R. L., W. A. Wood, and R. S. Emery. 1965. Lactate metabolism by Peptostreptococcus elsdenii: evidence for lactyl coenzyme A dehydrase. Biochem. Biophys. Acta 91:202.

Ballard, F. J., O. H. Filsell, and I. G. Jarrett. 1972. Effects of carbohydrate availability on lipogenesis in sheep. Biochem. J. 126:193.

Ballard, F. J., R. W. Hanson, and D. S. Kronfeld. 1969. Gluconeogenesis and lipogenesis in tissue from ruminants and nonruminant animals. Fed. Proc. 28:218.

Ballard, F. J. and I. T. Oliver. 1964. The effect of concentration on glucose phosphorylation and incorporation into glycogen in the livers of foetal and adult rats and sheep. Biochem. J. 92:131.

Barcroft, J., R. A. McAnally, and A. T. Phillipson. 1944. Absorption of volatile acids from the alimentary tract of the sheep and other animals. J. Exp. Biol. 20:120.

Barnard, E. A. 1969. Biological function of pancreatic ribonuclease. Nature 221:340.

Bassett, J. M. 1975. Dietary and gastro-intestinal control of hormones regulating carbohydrate metabolism in ruminants in Digestion and Metabolism in the Ruminant. I. W. McDonald and A.C.I. Warner (eds.), University of New England Publishing Unit, Armidale, Australia.

Bauchop, T. and S. R. Elsden. 1960. The growth of micro-organisms in relation to their energy supply. J. Gen. Micro. 23:457.

Beever, D. E., J. F. Coehlo Da Silva, and D. G. Armstrong. 1970. The effect of processing maize on its digestion in sheep. Proc. Nutr. Soc. 29:43A.

Benemann, J. R. and R. C. Valentine. 1971. High-energy electrons in bacteria. Adv. Microbiol. Physiol. 5:135.

Bentley, O. G., R. R. Johnson, T. V. Hershberger, J. H. Cline and A. L. Moxon. 1955. Cellulolytic-factor activity of certain short-chain fatty acids for rumen microorganisms in vitro. J. Nutr. 57:389.

Bentley, O. G., A. Lehmkuhl, R. R. Johnson, T. V. Hershberger, and A. L. Moxon. 1954. The "cellulolytic factor" activity of certain short-chained fatty acids. J. Am. Chem. Soc. 76:5000.

Bergen, W. G. and M. T. Yokoyama. 1977. Productive limits to rumen fermentation. J. Anim. Sci. (accepted for publication).

Bergman, E. N. 1973. Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Vet. 63:341.

Bergman, E. N. 1975. Production and utilization of metabolites by the alimentary tract as measured in portal and hepatic blood in Digestion and Metabolism in the Ruminant. I. W. McDonald and A.C.I. Warner (ed.), University of New England Publishing Unit, Armidale, Australia.

Berger, E. N. and J. E. Wolff. 1971. Metabolism of volatile fatty acids by liver and portal-drained viscera in sheep. Am. J. Physiol. 221:586.

Bergman, E. N., R. S. Reid, M. G. Murray, J. M. Brockway, and F. G. Whitelaw. 1965. Interconversions and production of volatile fatty acids in the sheep rumen. Biochem. J. 97:53.

Bhat, J. V. and H. A. Barker. 1947. Clostridium lacto-acetophilum nov. spec. and the role of acetic acid in the butyric acid fermentation of lactate. J. Bacteriol. 54:381.

Black, J. L. 1971. A theoretical consideration of the effect of preventing rumen fermentation on the efficiency of utilization of dietary energy and protein in lambs. Br. J. Nutr. 25:31.

Blaxter, K. L. 1962. The Energy Metabolism of Ruminants. Charles C. Thomas, Springfield, Illinois.

Blaxter, K. L. 1971. Methods of measuring the energy metabolism of animals and interpretation of results obtained. Fed. Proc. 30:1436.

Royce, W. E. and R. C. DiPrima. 1969. Elementary Differential Equations, J. Wiley and Sons, Inc., New York, 2nd edition.

Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Analyt. Biochem. 1:279.

Brill, W. J., E. A. Wolin, and R. S. Wolfe. 1964. Anaerobic formate oxidation: a ferredoxin dependent reaction. Science 144:297.

Brownell, G. L., M. Berman, and J. S. Robertson. 1968. Nomenclature for tracer kinetics. Int. J. Appl. Radiation Isotopes 19:249.

Bryant, M. P. and R. N. Doetsch. 1955. Factors necessary for the growth of Bacteroides succinogenes in the volatile acid fraction of rumen fluid. J. Dairy Sci. 38:340.

Buchanan-Smith, J. G., F. D. Horney, W. R. Usborne, and T. D. Burgess. 1973. Effects of feeding a zero- or high-roughage diet to cattle upon adipose tissue lipogenesis. Can. J. Physiol. Pharmacol. 51:532.

Bull, L. S., J. T. Reid, and D. E. Johnson. 1970. Energetics of sheep concerned with the utilization of acetic acid. J. Nutr. 100:262.

Burkhardt, J. D., L. B. Embry, and R. M. Luther. 1969. Corn grain processing methods for high concentrate beef cattle diets. J. Anim. Sci. 29:153 (Abstr.).

Castellana, F. S. and W. G. Kelly. 1973. Elution gradients generated under hydrostatic equilibrium employing various devices and solvents of different density. J. Chromatog. Sci. 11:429.

Christiansen, I. A., G. Hevesey, and S. Lomholt. 1924. Recherches, par une methode radiochimique, sur la circulation du bismuth dans 1' organisme. C. R. Hebd. Seanc. Acad. Sci. (Paris) 178:1324.

Church, D. C. 1969. Digestive Physiology and Nutrition of Ruminants. Vol. 1 - Digestive Physiology. Oregon State University Book Stores, Inc., Corvallis.

Clifford, A. J. and A. D. Tillman. 1968. Urea and isolated soybean protein in sheep purified diets. J. Anim. Sci. 27:484.

Cole, N. A. 1975. The Influence of Roughage Level and Corn Processing Method on the Site and Extent of Digestion by Beef Steers. Ph.D. thesis, Oklahoma State University.

Cole, N. A., R. R. Johnson, and F. N. Owens. 1976a. Influence of roughage level on the site and extent of digestion of whole shelled corn by beef steers. J. Anim. Sci. 43:483.

Cole, N. A., R. R. Johnson, and F. N. Owens. 1976b. Influence of roughage level and corn processing method on microbial protein synthesis by beef steers. J. Anim. Sci. 43:497.

Cook, R. M. and L. D. Miller. 1965. Utilization of volatile fatty acids in ruminants. I. Removal of them from portal blood by the liver. J. Dairy Sci. 48:1339.

Czerkawski, J. W. 1969. Methane production in ruminants and its significance. Wld. Rev. Nutr. Diet. 11:240.

Danielli, J. F., M. W. S. Hitchcock, R. A. Marshall, and A. T. Phillipson. 1945. The mechanism of absorption from the rumen as exemplified by the behavior of acetic, propionic and butyric acids. J. Exp. Biol. 22:75.

Davis, C. L. 1967. Acetate production in the rumen of cows fed either control or low-fiber, high-grain diets. J. Dairy Sci. 50:1621.

de Vries, W., W. M. C. van Wijch-Kapteyn, and S. K. H. Oosterhuis. 1974. The presence and function of cytochromes in Selenomonas ruminantium, Anaerovibrio lipolytica and Veillonella alcalescens. J. Gen. Microbiol. 81:69.

Demeyer, D. I. and C. J. Van Nevel. 1975. Methanogenesis, an integrated part of carbohydrate fermentation, and its control in Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner (eds.), University of New England Publishing Unit, Armidale, Australia.

Demeyer, D., H. Henderickx and C. Van Nevel. 1972. Evolution of a rumen fermentation balance, corrected for cell synthesis. Proc. Nutr. Soc. 31:54A.

Elsden, S. R. 1945. The fermentation of carbohydrates in the rumen of the sheep. J. Exp. Biol. 22:51.

Elsden, S. R. 1946. The application of the silica gel partition chromatogram to the estimation of volatile fatty acids. Biochem. J. 40:252.

el-Shazly, K. 1952. Degradation of protein in the rumen of the sheep. 1. Some volatile fatty acids, including branched-chain isomers, found in vivo. Biochem. J. 51:640.

Emmanuel, B., L. P. Milligan and B. V. Turner. 1974. The metabolism of acetate by rumen microorganisms. Can. J. Microbiol. 20:183.

Ensor, W. L., H. H. Olson, and V. F. Colenbrander. 1970. A report: committee on classification of particle size in feedstuffs. J. Dairy Sci. 53:689.

Esdale, W. J., G. A. Broderick, and L. D. Satter. 1968. Measurement of ruminal volatile fatty acid production from alfalfa hay or corn silage rations using a continuous infusion isotope dilution technique. J. Dairy Sci. 51:1823.

Evans, E. and J. G. Buchanan-Smith. 1975. Effects upon glucose metabolism of feeding a low- or high roughage diet at two levels of intake to sheep. Brit. J. Nutr. 33:33.

Faichney, G. J. 1975. The use of markers to partition digestion within the gastro-intestinal tract of ruminants in Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner (eds.), The University of New England Publishing Unit, Armidale, Australia.

Faires, R. A. and B. H. Parks. 1973. Radioisotope Laboratory Techniques. 4th ed. Holsted Press Division, John Wiley and Sons, Inc., New York, pp. 172-176.

Fell, B. F. and T. E. C. Weekes. 1975. Food intake as a mediator of adaptation in the ruminal epithelium in Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner (eds.), University of New England Publishing Unit, Armidale, Australia.

Freer, M. and R. C. Campling. 1965. Factors affecting the voluntary intake of food by cows. 7. The behavior and reticular motility of cows given diets of hay, dried grass, concentrates and ground, pelleted hay. Br. J. Nutr. 19:195.

Frobish, R. A. and C. L. Davis. 1977. Theory involving propionate and vitamin B_{12} in the low-milk fat syndrome. J. Dairy Sci. 60:268.

Galyean, M. L., D. G. Wagner, and R. R. Johnson. 1976. Site and extent of starch digestion in steers fed processed corn rations. J. Anim. Sci. 43:1088.

Garton, G. A., F. D. DeB. Hovell, and W. R. H. Duncan. 1972. Influence of dietary volatile fatty acids on the fatty acid triglycerides and special reference of the effect of propionate on the presence of branched chain components. Brit. J. Nutr. 28:409.

Gerken, H. J., M. B. Wise, R. W. Harvey, and E. R. Barrick. 1971. Whole corn, liquid supplements and polyethylene for beef cattle. J. Anim. Sci. 32:379 (Abstr.).

Gest, H. 1954. Oxidation and evolution of molecular hydrogen by microorganisms. Bact. Rev. 18:43.

Goldman, P., A. W. Alberts, and P. R. Vagelos. 1962. Requirement for a malonyl CoA-CO_2 exchange reaction in long chain but not

short chain fatty acid synthesis in Clostridium kluyveri. Biochem. Biophys. Res. Commun. 5:280.

Gowdy, C. M. and R. J. Mulholland. 1977. Sampling and transfer rate identification for tracer-kinetic systems. First International Conference on Mathematical Modeling, July, 1977, St. Louis, Mo.

Gray, F. V., G. B. Jones, and A. F. Pilgrim. 1960. The rates of production of volatile fatty acids in the rumen. Aust. J. Agric. Res. 11:383.

Gray, F. V., A. F. Pilgrim, H. J. Rodda, and R. A. Weller. 1951. Fermentation in the rumen of the sheep. IV. The nature and origin of the volatile fatty acids in the rumen of the sheep. J. Exp. Biol. 29:57.

Gray, F. V., A. F. Pilgrim, H. J. Rodda, and R. A. Weller. 1951. Volatile fatty acids in the rumen of the sheep. Nature, Lond. 167:954.

Gray, F. V., R. A. Weller, and G. B. Jones. 1965. The rates of production of volatile fatty acids in the rumen. II. Measurement of production in an artificial rumen and application of the isotope dilution technique to the rumen of a sheep. Aust. J. Agric. Res. 16:145.

Gray, F. V., R. A. Weller, A. F. Pilgrim, and G. B. Jones. 1962. A stringent test for the artificial rumen. Aust. J. Agric. Res. 13:343.

Gray, F. V., R. A. Weller, A. F. Pilgrim, and G. B. Jones. 1966. The rates of production of volatile fatty acids in the rumen. III. Measurement of production in vivo by two isotope dilution procedures. Aust. J. Agric. Res. 17:69.

Gunsalus, I. C. and C. W. Schuster. 1961. Energy-yielding metabolism in bacteria in The Bacteria, Vol. 2. I. C. Gunsalus and R. Y. Stanier (eds.), Academic Press, New York.

Hanson, R. W. and F. J. Ballard. 1967. The relative significance of acetate and glucose as precursors for lipid synthesis in liver and adipose tissue from ruminants. Biochem. J. 105:529.

Henderson, H. E. and M. R. Geasler. 1971. Physical preparation of corn grain for feedlot cattle. Cooperative Extension Service Bulletin AH-BC-71, Michigan State University.

Hetenyi, G. and K. H. Norwich. 1974. Validity of the rates of production and utilization of metabolites as determined by tracer methods in intact animals. Fed. Proc. 33:1841.

Hevesey, G. 1923. The absorption and translocation of lead by plants. Biochem. J. 17:439.

Hinman, D. D. and R. R. Johnson. 1974. Influence of processing methods on digestion of sorghum starch in high concentrate beef cattle rations. J. Anim. Sci. 39:417.

Hishinuma, F., S. Kanegasaki, and H. Takahashi. 1968. Ruminal fermentation and sugar concentrations: A model experiment with Selenomonas ruminantium. Agr. Biol. Chem. 32:1325.

Hixon, D. C., E. E. Hatfield, and P. E. Lamb. 1969. Comparison of whole shelled corn with cracked corn in cattle finishing diets. J. Anim. Sci. 29:161 (Abstr.).

Hobson, P. N. 1965. Continuous culture of some anaerobic and faculatively anaerobic rumen bacteria. J. Gen. Microbiol. 38:167.

Hobson, P. N. 1972. Physiological characteristics of rumen microbes and relation to diet and fermentation patterns. Proc. Nutr. Soc. 31:135.

Hobson, P. N. and M. MacPherson. 1952. Amylases of Clostridium butyricum and a Streptococcus isolated from the rumen of sheep. Biochem. J. 52:671.

Hobson, P. N. and R. Summers. 1967. The continuous culture of anaerobic bacteria. J. Gen. Microbiol. 47:53.

Hodgson, J. C. and P. C. Thomas. 1975. A relationship between the molar proportion of propionic acid and the clearance rate of the liquid phase in the rumen of the sheep. Br. J. Nutr. 33:447.

Hogan, J. P. and R. H. Weston. 1970. Quantitative aspects of microbial protein synthesis in the rumen in Physiology of Digestion and Metabolism in the Ruminant. A. J. Phillipson (ed.), Oriel Press, Newcastle upon Tyne, England.

Horsfield, S., J. M. Infield, and E. F. Annison. 1974. Compartmental analysis and model building in the study of glucose kinetics in the lactating cow. Proc. Nutr. Soc. 33:9.

Hungate, R. E. 1963a. Symbiotic associations: the rumen bacteria. Symposia of the Society for General Microbiol. 13:266.

Hungate, R. E. 1963b. Polysaccharide storage and growth efficiency in Ruminococcus albus. J. Bact. 86:848.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press, New York.

Hungate, R. E. 1967. Hydrogen as an intermediate in the rumen fermentation. Archiv. für Mikrobiologie 59:158.

Hungate, R. E. 1975. The rumen microbial ecosystem. Ann. Rev. Ecol. and Systematics 6:39.

Hungate, R. E., R. A. Mak, and M. Simesen. 1961. Rates of production of individual volatile fatty acids in the rumen of lactating cows. Appl. Microbiol. 9:554.

Hungate, R. E., J. Reiche, and R. Prins. 1971. Parameters of rumen fermentation in a continuously fed sheep: evidence of a microbial rumination pool. App. Microbiol. 22:1104.

Hyden, S. 1961. The use of reference substances and the measurement of flow in the alimentary tract in Digestive Physiology and Nutrition of the Ruminant. D. Lewis (ed.), Butterworths, London, p. 35.

IBM. 1969. System/360 Continuous System Modeling Program Manual. Version 1.3. IBM Corporation, White Plains, New York.

Isaacson, H. R., F. C. Hinds, M. P. Bryant, and F. N. Owens. 1975. Efficiency of energy utilization by mixed rumen bacteria in continuous culture. S. Dairy Sci. 58:1645.

Jacquez, J. A. 1972. Compartmental Analysis in Biology and Medicine. Elsevier Publishing Company, Amsterdam.

Jayasuriya, G. C. N. and R. E. Hungate. 1959. Lactate conversions in the bovine rumen. Arch. Biochem. Biophys. 82:274.

Jesse, G. W., G. B. Thompson, and J. L. Clark. 1976a. Net energy values of corn and corn silage fed in various combinations to steers slaughtered at 341, 454 and 545 kg. J. Anim. Sci. 43:1044.

Jesse, G. W., G. B. Thompson, J. L. Clark, K. G. Weimer, and D. P. Hutcheson. 1976b. Effects of various ratios of corn and corn silage and slaughter weight on the performance of steers individually fed. J. Anim. Sci. 43:1049.

Johnson, R. R., E. T. Clemens, D. D. Hinman, N. A. Cole, and D. Williams. 1974. Influence of grain processing on development of subclinical acidosis in beef cattle. Okla. Agr. Expt. Sta. Report MP-92.

Jones, G. B. 1965. Determination of the specific activity of labeled blood glucose by liquid scintillation using glucose pentaacetate. Anal. Biochem. 12:249.

Joyner, A. E. and R. L. Baldwin. 1966. Enzymatic studies of pure cultures of rumen microorganisms. J. Bacteriol. 92:1321.

Judson, G. J. and R. A. Leng. 1973a. Studies on the control of gluconeogenesis in sheep: effect of glucose infusion. Brit.

J. Nutr. 21:159.

Judson, G. J. and R. A. Leng. 1973b. Studies on the control of gluconeogenesis in sheep: Effect of propionate, casein and butyrate infusions. Brit. J. Nutr. 21:175.

Judson, G. J., E. Anderson, J. R. Luick, and R. A. Leng. 1968. The contribution of propionate to glucose synthesis in sheep given diets of different grain content. Brit. J. Nutr. 22:69.

Karr, M. R., C. O. Little, and G. E. Mitchell, Jr. 1966. Starch disappearance from different segments of the digestive tract of steers. J. Anim. Sci. 25:652.

Katz, J. and A. Dunn. 1967. Glucose-2-T as a tracer for glucose metabolism. Biochemistry 6:1.

Kiddle, P., R. A. Marshall, and A. T. Phillipson. 1951. A comparison of the mixtures of acetic, propionic and butyric acids in the rumen and in the blood leaving the rumen. J. Physiol. 113:207.

Kistner, A. 1965. Possible factors influencing the balance of different species of cellulolytic bacteria in the rumen in Physiology of Digestion in the Ruminant. R. W. Dougherty (ed.), Butterworths, Washington, D. C.

Kleiber, M. 1967. An old professor of animal husbandry ruminates. Ann. Rev. Physiol. 29:1.

Kleiber, M. 1975. Metabolic turnover rate: A physiological meaning of the metabolic rate per unit body weight. J. Theor. Biol. 53:199.

Knight, W. M. 1971. An Evaluation of Intraruminal Urea Infusion for Growing Lambs. Ph.D. Thesis, University of Illinois, Urbana.

Knox, K. L., A. L. Black, and M. Kleiber. 1967. Some kinetic characteristics of rumen short-chain fatty acids as measured by the isotope dilution method. J. Dairy Sci. 50:1716.

Krebs, H. A. and J. M. Lowenstein. 1960. The tricarboxylic acid cycle in D. M. Greenberg (ed.), Metabolic Pathways. Volume 1. Academic Press, New York.

Kristensen, S. 1974. Ruminal biosynthesis of aromatic amino acids from arylacetic acids, glucose, shikimic acid and phenol. Brit. J. Nutr. 31:357.

Kropp, J. R. 1975. Rumen Microbial Protein Synthesis from Urea When Fed with Low Quality Roughage Rations. Ph.D. Thesis, Oklahoma State University.

Lawlor, M. J., D. Giesecke, and K. Walser-Kärst. 1966. Comparative studies on the digestive physiology of sheep fed on semi-purified or roughage-concentrate diets. 1. Food and water intake, rumen volume and rates of parotid secretion. Brit. J. Nutr. 20:373.

Leng, R. A. 1970a. Formation and production of volatile fatty acids in the rumen in Physiology of Digestion and Metabolism in the Ruminant. A. T. Phillipson (ed.), Oriel Press, Newcastle upon Tyne, England.

Leng, R. A. 1970b. Glucose synthesis in ruminants. Adv. Vet. Sci. Comp. Med. 14:209.

Leng, R. A. and D. J. Brett. 1966. Simultaneous measurements of the rates of production of acetic, propionic and butyric acids in the rumen of sheep on different diets and the correlation between production rates and concentrations of these acids in the rumen. Brit. J. Nutr. 20:541.

Leng, R. A. and G. J. Leonard. 1965. Measurement of the rates of production of acetic, propionic and butyric acids in the rumen of sheep. Brit. J. Nutr. 19:469.

Leng, R. A., J. W. Steel, and J. R. Luick. 1967. Contribution of propionate to glucose synthesis in sheep. Biochem. J. 103:785.

Leng, R. A., J. L. Corbett, and D. J. Brett. 1968. Rates of production of volatile fatty acids in the rumen of grazing sheep and their relation to ruminal concentrations. Brit. J. Nutr. 22:57.

Luick, J. R., S. J. Person, R. D. Cameron, and R. G. White. 1973. Seasonal variations in glucose metabolism of reindeer (Rongifer torandus L.) estimated with U-^{14}C-glucose and 3-^{3}H-glucose. Brit. J. Nutr. 29:245.

Madsen, A. and P. Willeberg (ed.). 1972. Proceedings of the International Summer School on Computers and Research in Animal Nutrition and Veterinary Medicine. Fredericksberg Bogtrykkeri, Copenhagen.

Mahler, H. R. and E. H. Cordes. 1971. Biological Chemistry. Harper and Row, New York.

Mangold, E. 1934. The digestion and utilization of crude fiber. Nutr. Abst. Rev. 3:647.

Massey, L. K., J. R. Sokatch, and R. S. Conrad. 1976. Branched-chain amino acid catabolism in bacteria. Bact. Rev. 40:42.

Mohler, R. R. 1973. Bilinear Control Processes. Academic Press, New York.

Morris, J. A. 1975. The physiology of obligate anaerobiosis. Adv. Microbiol. Physiol. 12:169.

Mortenson, L. E., R. C. Valentine, and J. E. Carnahan. 1963. Ferrodoxin in the phosphoroclastic reaction of pyruvic acid and its relation to nitrogen fixation in Clostridium pasteurianum. J. Biol. Chem. 238:794.

Moyle, V., E. Baldwin, and R. Scarisbrick. 1948. Separation and estimation of saturated C_2-C_8 fatty acids by buffered partition columns. Biochem. J. 43:308.

Mulholland, R. J. 1976. Personal Communication.

Mulholland, R. J. and C. M. Gowdy. 1977. Theory and application of the measurement of structure and determination of function for laboratory ecosystems. J. Theor. Biol. (accepted).

Mulholland, R. J. and M. S. Keener. 1974. Analysis of linear compartment models for ecosystems. J. Theor. Biol. 44:105.

Murray, M. G., R. S. Reid, and T. M. Sutherland. 1962. The rate of passage of digesta from the reticulo-rumen of the sheep. J. Physiol. 164:26P.

McCormick, N. G., Z. J. Ordal, and H. R. Whiteley. 1962. Degradation of pyruvate by Micrococcus lactilyticus. I. General properties of the formate-exchange reaction. J. Bact. 83:887.

McCullough, M. W. and J. K. Matsushima. 1973. Effect of corn processing on starch digestibility. J. Anim. Sci. 37:350 (Abstr.).

Nasr, H. 1950. Amylolytic activity in the rumen of the sheep. J. Agric. Sci., Camb. 40:308.

Neudoerffer, T. S., D. R. McLaughlin, S. J. Slinger, and F. D. Horney. 1973. Difficulties associated with the use of tritiated polyethylene glycol as a water-soluble digestion marker in ruminants. J. Anim. Sci. 36:749.

Nordin, M. and R. C. Campling. 1976. Digestibility studies with cows given whole and rolled cereal grains. Anim. Prod. 23:305.

O'Neal, R. M., R. E. Koeppe, and E. I. Williams. 1966. Utilization in vivo of glucose and volatile fatty acids by sheep brain for the synthesis of acidic amino acids. Biochem. J. 101:591.

Ørskov, E. R. 1975. Manipulation of rumen fermentation for maximum food utilization. Wld. Rev. Nutr. Diet. 22:152.

Ørskov, E. R. 1977. Composition of absorbed nutrients and animal metabolism. J. Anim. Sci. (accepted for publication).

Ørskov, E. R. and D. M. Allen. 1966. Utilization of salts of volatile fatty acids by growing sheep. I. Acetate, propionate and butyrate as sources of energy for young growing lambs. Brit. J. Nutr. 20:295.

Ørskov, E. R., F. D. De B. Hovell, and D. M. Allen. 1966. Utilization of salts of volatile fatty acids by growing sheep. II. Effect of stage of maturity and hormone implantation on the utilization of volatile fatty acid salts as sources of energy for growth and fattening. Brit. J. Nutr. 20:307.

Ørskov, E. R., W. P. Flatt, and P. W. Moe. 1968. Fermentation balance approach to estimate extent of fermentation and efficiency of volatile fatty acid formation in ruminants. J. Dairy Sci. 51:1429.

Ørskov, E. R., C. Fraser, and J. G. Gordon. 1974. Effect of processing of cereals on rumen fermentation, digestibility, rumination time, and firmness of subcutaneous fat in lambs. Brit. J. Nutr. 32:59.

Ottenstein, D. M. and D. A. Bartley. 1971. Separation of free acids C_2-C_5 in dilute aqueous solution column technology. J. Chromatog. Sci. 9:673.

Palmquist, D. L. and R. L. Baldwin. 1966. Enzymatic techniques for the study of pathways of carbohydrate utilization in the rumen. Appl. Microbiol. 14:60.

Pease, M. C. 1965. Methods of Matrix Algebra. Academic Press, New York.

Pennington, R. J. 1952. The metabolism of short-chain fatty acids in the sheep. 1. Fatty acid utilization and ketone body production by rumen epithelium and other tissues. Biochem. J. 51:251.

Pennington, R. J. 1954. The metabolism of short-chain fatty acids in the sheep. 2. Further studies with rumen epithelium. Biochem. J. 56:410.

Peterson, E. A. and H. A. Sobev. 1959. Variable gradient device for chromatography. Anal. Chem. 31:857.

Phillipson, A. T. and R. A. McAnally. 1942. Studies on the fate of carbohydrates in the rumen of the sheep. J. Exp. Biol. 19:199.

Poole, D. A. and D. M. Allen. 1970. Utilization of salts of volatile fatty acids by growing sheep. V. Effect of type of fermentation of the basal diet on the utilization of salts of acetic acid by growing sheep. Brit. J. Nutr. 24:695.

Putnam, P. A., R. Lehmann, and R. E. Davis. 1966a. Feed intake and salivary secretion by steers. J. Anim. Sci. 25:817.

Putnam, P. A., D. A. Yarns, and R. E. Davis. 1966b. Effect of pelleting rations and hay:grain ratio on salivary secretions and ruminal characteristics of steers. J. Anim. Sci. 25:1176.

Purser, D. B. and S. M. Buechler. 1966. Amino acid composition of rumen organisms. J. Dairy Sci. 49:81.

Reilly, P. E. B. and E. J. H. Ford. 1971. The effects of different dietary contents of protein on amino acid and glucose production and on the contribution of amino acids to gluconeogenesis in sheep. Brit. J. Nutr. 26:249.

Rescigno, A. and J. S. Beck. 1972. Compartments. in R. Rosen (ed.), Foundations of Mathematical Biology: Cellular Systems. Vol. II. Academic Press, New York.

Riggs, D. S. 1976. Control Theory and Physiological Feedback Mechanisms. R. E. Krieger Publishing Co., Huntington, New York.

Ross, J. P. and W. D. Kitts. 1973. Relationship between post-prandial plasma volatile fatty acids, glucose and insulin levels in sheep fed different feeds. J. Nutr. 103:488.

Rounds, P. W. 1973. The Effect of the Physical Form of Corn on Net Energy Value, Feedlot Performance and Carcass Merit of Beef Steers. M.S. Thesis, Oklahoma State University.

Satter, L. D. and W. J. Esdale. 1968. In vitro lactate metabolism by ruminal ingesta. Appl. Microbiol. 16:680.

Satter, L. D., J. W. Suttie, and B. R. Baumgardt. 1967. Influence of accompanying substrate on in vitro volatile fatty acid formation from α-cellulose-^{14}C. J. Dairy Sci. 50:1626.

Sauer, F. D., J. D. Erfle, and S. Mahadevan. 1975. Amino acid biosynthesis in mixed rumen cultures. Biochem. J. 150:357.

Schake, L. M. and J. K. Riggs. 1966. Rumination patterns of confined beef cows at three levels of feed intake. J. Anim. Sci. 25:248 (Abstr.).

Schwartz, H. M. and F. M. C. Gilchrist. 1975. Microbial interactions with the diet and the host animal in Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner (ed.), The University of New England Publishing Unit, Armidale, Australia.

Shaw, J. C. 1961. Techniques for studying rumen metabolism with special reference to the use of radio-isotopes in Digestive Physiology and Nutrition of the Ruminant (D. Lewis, editor). London: Butterworths. p. 167.

Sheppard, A. J., R. M. Forbes, and B. C. Johnson. 1959. Rate of acetic acid in production in the rumen determined by isotope

dilution. P. S. E. B. M. 101:715.

Sheppard, C. W. 1948. The theory of the study of transfers within a multi-compartment system using isotopic tracers. J. Appl. Phys. 19:70.

Sheppard, C. W. 1962. Basic Principles of the Tracer Method: Introduction to Mathematical Tracer Kinetics. John Wiley and Sons, Inc., New York.

Shipley, R. A. and R. E. Clark. 1972. Tracer Methods for in vivo Kinetics: Theory and Applications. Academic Press, New York.

Smith, G. H. 1971. Glucose metabolism in the ruminant. Proc. Nutr. Soc. 30:265.

Smith, R. M., W. S. Osborne-White and G. R. Russell. 1967. Metabolism of propionate by sheep liver. Pathway of propionate metabolism in aged homogenate and mitochondria. Biochem. J. 104:441.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods (6th ed.). Iowa State University Press, Ames, Iowa.

Stadtman, E. R., T. C. Stadtman and H. A. Barker. 1949. Tracer experiments on the mechanism of synthesis of valeric and caproic acids by Clostridium kluyveri. J. Biol. Chem. 178:677.

Steel, J. W. and R. A. Leng. 1973a. Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 1. The kinetics of glucose metabolism. Brit. J. Nutr. 30:451.

Steel, J. W. and R. A. Leng. 1973b. Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 2. Synthesis of glucose from ruminal propionate. Brit. J. Nutr. 30:475.

Stevens, C. E. 1970. Fatty acid transport through the rumen epithelium in Physiology of Digestion and Metabolism in the Ruminant. A. T. Phillipson (ed.), Oriel Press, Newcastle upon Tyne, England.

Stevens, C. E. and B. K. Stettler. 1966. Factors affecting the transport of volatile fatty acids across rumen epithelium. Am. J. Physiol. 210:365.

Stouthamer, A. H. and C. Bettenhaussen. 1973. Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. Biochim. Biophys. Acta 301:53.

Stewart, G. N. 1897. Researches on the circulation time and on the influences which affect it. J. Physiol. 22:159.

Sutton, J. D. 1971a. Carbohydrate digestion and glucose supply in the gut of the ruminant. Proc. Nutr. Soc. 30:243.

Sutton, J. D. 1971b. The rate of carbohydrate fermentation in the rumen. Proc. Nutr. Soc. 30:36.

Sutton, J. D. 1972. In vivo measurement of energy supply and protein synthesis in the rumen in Tracer Studies on Non-protein Nitrogen for Ruminants. Intn'l. Atomic Energy Agency, Vienna.

Swick, R. W. 1962. Propionic acid metabolism: mechanism of the methylmalonyl isomerase reaction and the reduction of acrylyl coenzyme A to propionyl coenzyme A in Propionibacteria. Proc. Nat'l. Acad. Sci., U.S.A. 48:288.

Tappeiner, H. 1884. Untersuchungen über die Gärung der Cellulose insbesondere über deren Lösung in darmkanale. Z. Biol. 20:52.

Thomas, G. J. 1960. Metabolism of the soluble carbohydrates of grasses in the rumen of the sheep. J. Agric. Sci., Camb. 54:360.

Thomson, D. J. 1972. Physical form of the diet in relation to rumen fermentation. Proc. Nutr. Soc. 31:127.

Thompson, E. H. 1969. An Introduction to the Algebra of Matrices with Some Applications. Adam Hilger, London.

Thompson, J. K. and P. N. Hobson. 1971. Polysaccharide synthesis and degradation by rumen micro-organisms in vitro. J. Agric. Sci., Camb. 76:423.

Tropper, A. M. 1962. Matrix Theory for Electrical Engineers. Addison-Wesley Publishing Company, Inc., Reading, Mass.

Topps, J. H., R. N. B. Kay, E. D. Goodall, F. G. Whitelaw, and R. S. Reid. 1968. Digestion of concentrate and of hay diets in the stomach and intestines of ruminants. 2. Young steer. Brit. J. Nutr. 22:281.

Tucker, R. E., G. E. Mitchell, Jr., and C. O. Little. 1968. Ruminal and postruminal starch digestion in sheep. J. Anim. Sci. 27:824.

Turnbull, H. W. and A. C. Aitken. 1952. An Introduction to the Theory of Canonical Matrices. Blackie and Son Limited, London.

Ulyatt, M. J., F. G. Whitelaw, and F. G. Watson. 1970. The effect of diet on glucose entry rates in sheep. J. Agric. Sci., Camb., 75:565.

Utley, P. R. and W. C. McCormick. 1975. Comparison of cattle finishing diets containing various physical forms of corn. J. Anim. Sci. 40:952.

Vance, R. D., R. R. Johnson, E. W. Klosterman, B. A. Dehority, and R. L. Preston. 1970. All-concentrate rations for growing-finishing cattle. Ohio Agricultural Research and Development

Center, Research Summary 43.

Vance, R. D., R. S. Preston, E. W. Klosterman, and V. R. Cahill. 1972. Utilization of whole shelled corn and crimped corn grain with varying proportions of corn silage by growing-finishing steers. J. Anim. Sci. 35:598.

Waldo, D. R. 1973. Extent and partition of cereal grain starch digestion in ruminants. J. Anim. Sci. 37:1062.

Waldo, D. R., J. E. Keys, Jr. and C. H. Gordon. 1971. Corn starch digestion in the bovine. J. Anim. Sci. 33:304.

Walker, D. J. 1965. Energy metabolism in rumen microorganisms in Physiology of Digestion in the Ruminant. R. W. Dougherty (ed.), Butterworths, Washington, D.C.

Walker, D. J. 1968. Energy utilization for polysaccharide synthesis by mixed rumen organisms fermentating soluble carbohydrates. App. Microbiol. 16:1672.

Walker, D. J. and P. R. Monk. 1971. Fate of carbon passing through the glucose pool of rumen digesta. Appl. Microbiol. 22:741.

Walker, D. M. 1959. The development of the digestive system of the young animal. III. Carbohydrase enzyme development in the young lamb. J. Agric. Sci., Camb. 53:374.

Wallnöfer, P., R. L. Baldwin, and E. Stagno. 1966. Conversion of ^{14}C-labeled substrates to volatile fatty acids by the rumen microbiota. Appl. Microbiol. 14:1004.

Warner, A. C. I. 1964. Production of volatile fatty acids in the rumen: methods of measurement. Nutr. Abstr. Rev. 34:339.

Webster, A. J. F. 1974. Clearance by the hepatic portal circulation of the products of digestion and metabolism in the ruminant gut. Proc. Nutr. Soc. 33:155.

Weekes, T. E. C. and A. J. F. Webster. 1974. Metabolism of propionate by the portal-drained viscera in sheep. Proc. Nutr. Soc. 33:71A.

Weichental, B. A. and R. J. Webb. 1969. Whole or ground shelled corn and hormone treatments for yearling steers. Beef Day Report, Univ. of Illinois.

Weigand, E., J. W. Young, and A. D. McGilliard. 1972. Extent of propionate metabolism during absorption from the bovine rumino-reticulum. Biochem. J. 126:201.

Weigand, E., J. W. Young, and A. D. McGilliard. 1975. Volatile fatty acid metabolism by rumen mucosa from cattle fed hay or grain. J. Dairy Sci. 58:1294.

Weller, R. A., F. V. Gray, A. F. Pilgrim, and G. B. Jones. 1967. The rates of production of volatile fatty acids in the rumen. IV. Individual and total volatile fatty acids. Aust. J. Agric. Res. 18:107.

Weller, R. A., A. F. Pilgrim, and F. V. Gray. 1969. Volatile fatty acid production in the rumen of the grazing sheep: Its use as an indicator of pasture value. Brit. J. Nutr. 23:97.

Weston, R. H. and J. P. Hogan. 1968. The digestion of pasture plants by sheep. 1. Ruminal production of volatile fatty acids by sheep offered diets of ryegrass and forage oats. Aust. J. Agric. Res. 19:419.

White, R. G., J. W. Steel, R. A. Leng, and J. R. Luick. 1969. Evaluation of three isotope-dilution techniques for studying the kinetics of glucose metabolism in sheep. Biochem. J. 114:203.

White, T. W., F. G. Hembry, and W. L. Reynolds. 1972. Influence of grinding, supplemental nitrogen source and roughage on the digestibility of corn. J. Anim. Sci. 34:672.

White, T. W., W. L. Reynolds, and F. G. Hembry. 1975a. Comparison of supplements containing soybean meal and urea fed with whole or ground corn to beef cattle. J. Anim. Sci. 40:1.

White, T. W., F. G. Hembry, and W. L. Reynolds. 1975b. Influence of feeding rice straw and urea supplement with whole and ground corn on feedlot performance, rate of passage and digestibility by steers. J. Anim. Sci. 41:1180.

Wiberg, D. M. 1971. State Space and Linear Systems. Schaum's Outline Series. McGraw-Hill Book Company, New York.

Wiltrout, D. W. and L. D. Satter. 1972. Contribution of propionate to glucose synthesis in the lactating and nonlactating cow. J. Dairy Sci. 55:307.

Wolfe, R. S. 1971. Microbial formation of methane. Adv. Microbiol. Physiol. 6:107.

Wolfe, R. S. and D. J. O'Kane. 1955. Cofactors of the carbon dioxide exchange reaction of Clostridium butyricum. J. Biol. Chem. 215:637.

Wolin, M. J. 1960. A theoretical fermentation balance. J. Dairy Sci. 43:1952.

Wolin, M. J. 1974. Metabolic interactions among intestinal microorganisms. Am. J. Clin. Nutr. 27:1320.

Wolin, E. A., M. J. Wolin, and R. S. Wolfe. 1963. Formation of methane by bacterial extracts. J. Biol. Chem. 238:2882.

Wood, J. W. and R. S. Wolfe. 1966a. Alkylation of an enzyme in the methane-forming system of Methanobacillus omelianskii. Biochem. Biophys. Res. Commun. 22:119.

Wood, J. W. and R. S. Wolfe. 1966b. Components required for the formation of methane from methyl-cobalamin by extracts of Methanobacillus omelianskii. J. Bact. 92:696.

Young, J. W. 1977. Gluconeogenesis in cattle: significance and methodology. J. Dairy Sci. 60:1.

APPENDIX A

SUPPLEMENTARY CALCULATIONS ASSOCIATED WITH THE ERROR PROPAGATION ALGORITHM

Eigenvalues of the (EM^{-1}) Matrix

If the absolute values of the eigenvalues of $\underline{EM}^{-1}$ are < 1, then the infinite series in Equation (2.52) will converge. Consider the mean ($\underline{M}$) and error ($\underline{E}$) matrices associated with the 608-WC animal-ration period listed in Table XXXIX where

$$\underline{M} = \begin{bmatrix} 39.80 & 1.35 & 6.04 \\ 2.91 & 59.00 & 1.83 \\ 18.30 & 0.42 & 60.10 \end{bmatrix} \tag{A.1}$$

and

$$\underline{E} = \begin{bmatrix} 1.11 & 0.13 & 0.41 \\ 0.21 & 1.09 & 0.19 \\ 0.59 & 0.09 & 2.06 \end{bmatrix} . \tag{A.2}$$

Therefore,

$$\underline{EM}^{-1} = \begin{bmatrix} 0.0258539 & 0.0015821 & 0.0086805 \\ 0.0028680 & 0.0183925 & 0.0013125 \\ -0.0010623 & 0.0013053 & 0.0343432 \end{bmatrix} . \tag{A.3}$$

The characteristic equation for this particular matrix is

$$-s^3 + 0.07858958\ s^2 - 0.00199791\ s + 0.000016328 = 0\ . \qquad (A.4)$$

The eigenvalues corresponding to Equation (A.4) include one real root and two complex roots. These eigenvalues are

$$s_1 = 0.0179787 \qquad (A.5)$$

$$s_2 = 0.0482842 + 0.0374529\ j \qquad (A.6)$$

$$s_3 = 0.0482842 - 0.0374529\ j\ . \qquad (A.7)$$

Clearly,

$$\left|s_i\right| < 1 \qquad (A.8)$$

and, by definition, the infinite series in Equation (2.52) will converge.

Consideration of Higher Order Terms in Equation (2.52)

Consideration of only first order terms of Equation (2.52) leads to the following solution set for (A.1) and (A.2):

$$\underline{F}_m = \begin{bmatrix} -0.0527583 & 0.0011697 & 0.0052666 \\ 0.0021043 & -0.0339523 & 0.0008223 \\ 0.0161098 & -0.0001189 & -0.0348872 \end{bmatrix} \qquad (A.9)$$

$$\underline{F}_e = \begin{bmatrix} -0.0013663 & -0.0000551 & -0.0002756 \\ -0.0000438 & -0.0006201 & -0.0000019 \\ 0.0004532 & -0.0000222 & -0.0010585 \end{bmatrix}\ . \qquad (A.10)$$

When the second order term $(EM^{-1})^2$ in Equation (2.52) is included in the calculations, the percentage increase in $\underline{F}_e$ due to the inclusion of this additional term is given by

$$\%\underline{\Delta} = \begin{bmatrix} 2.6 & 6.4 & 7.8 \\ 6.7 & 1.9 & 5.9 \\ 2.8 & 4.8 & 3.1 \end{bmatrix} \tag{A.11}$$

where

$$\%\underline{\Delta} = \left[1 - \left[\frac{f_{e_1} + f_{e_2}}{f_{e_1}} \right] \right] \times 100 \, . \tag{A.12}$$

Generally, the error incurred by not including higher order terms is less than 10%, which may be acceptable for most biological applications. However, the inclusion of the second order term may be desirable if additional precision is required.

APPENDIX B

APPLICABILITY OF FERMENTATION BALANCE CALCULATIONS

The extent of fermentation in the rumen of steers consuming a high-concentrate whole corn diet will be evaluated using the fermentation balance approach developed in Chapter III. Data to be considered were obtained from the experiments of Rounds (1973) and Cole et al. (1976a,b) and are presented in Table LII. Steers were fed hourly a 12.5% CP ration containing 82-84% whole corn, 7% cottonseed hulls and a protein supplement.

Appropriate values for A,P,B,V and C from Table LII into Equation (3.31) yields the number of moles of hexose fermented per mole of total VFA produced such that

$$H_x = 0.340 + 0.073 + 0.132 + 0.031 + 0.018$$
$$= 0.594 \text{ moles hexose per mole VFA.}$$

The proportion of hexose converted to acetate, propionate, butyrate, valerate or caproate is calculated from Equation (3.32)-(3.36) where a = 0.572, p = 0.123, b = 0.222, v = 0.052 and c = 0.031, respectively. The number of moles of hexose degraded in the rumen per day can be calculated by dividing the daily ruminal starch digestion (2110 g) by the average molecular weight (162 g/mole) of hexose in starch. Therefore, 13.0 moles of hexose per day were fermented in the rumen.

TABLE LII

PARAMETERS USED IN THE FERMENTATION BALANCE CALCULATIONS FROM ROUNDS (1973) AND COLE ET AL. (1976a,b)

Parameter	Units	Value
VFA molar %		
Acetic (A)	%	67.9
Propionic (P)	%	14.6
Butyric (B)	%	13.2
Valeric (V)	%	3.1
Caproic (C)	%	1.2
DM Intake	g/d	5230.0
Starch Intake	g/d	3140.0
Ruminal DM Digestion	g/d	2442.0
Ruminal Starch Digestion	g/d	2110.0
Methane Production	moles/kg DMI	0.567
Microbial Nitrogen	g/d	40.3

Consequently, the total moles of VFA produced per day can be calculated as follows:

$$(0.572) \times (13.0) = 7.44 \text{ moles hexose} \longrightarrow 14.88 \text{ moles Ac}$$

$$(0.123) \times (13.0) = 1.60 \text{ moles hexose} \longrightarrow 3.20 \text{ moles Pr}$$

$$(0.222) \times (13.0) = 2.89 \text{ moles hexose} \longrightarrow 2.89 \text{ moles Bu}$$

$$(0.052) \times (13.0) = 0.68 \text{ moles hexose} \longrightarrow 0.68 \text{ moles V}$$

$$(0.031) \times (13.0) = 0.40 \text{ moles hexose} \longrightarrow 0.27 \text{ moles C}$$

$$\Sigma = 21.9 \text{ moles VFA}$$

The theoretical number of moles of methane and carbon dioxide per mole of hexose fermented can easily be calculated from Equation (3.39a) and Equation (3.41), respectively. These calculations yield values of 0.618 moles CH_4 and 1.08 moles CO_2 per mole of hexose fermented.

The balance equation for the total hexose fermentation becomes

$$13.0 \text{ hexose} \longrightarrow 14.9 \text{ Ac} + 3.2 \text{ Pr} + 2.89 \text{ Bu} + 0.68 \text{ V} + 0.27 \text{ C} + 8.03 \text{ } CH_4 + 14.0 \text{ } CO_2.$$

Consideration of Equation (3.43) reveals that 2366 kJ of energy is lost during the fermentation as heat (RHF). This amounts to 6.46% of the total energy in hexose. On the other hand, the efficacy of hexose conversion to VFAs is 74.1% as calculated from Equation (3.44).

If the values for methane production obtained by Rounds (1973) apply to the experiments of Cole et al. (1976a,b), then 2.97 moles of methane were actually produced from the fermentation of 13.0 moles of hexose. Energetically this accounts for only 37% of the theoretical methane yield and 7.16% of the total fermentable hexose energy.

Assuming that this discrepancy is entirely due to cell synthesis, then 4467 kJ are available for cell synthesis which accounts for 12.2% of the energy in hexose.

The minimum yield of ATP per mole of hexose fermented from this fermentation can be calculated from Equation (3.22) where $\theta = 0.37$. This assumption may be valid since steers consuming similar rations exhibited rumen dilution rates approaching 14%/hr (Chapter V). A shift to less efficient metabolic pathways might be expected under such growth conditions. Therefore, 3.8 moles ATP are generated per mole hexose fermented or 49.4 moles ATP derived from the total fermentation.

Cole et al. (1976b) reported 40.3 g of microbial nitrogen flowing through the abomasum per day. This amounts to 403 g cells per day assuming a bacterial nitrogen content of 10% (Hungate, 1966). Isaacson et al. (1975) reported that 6.24 mmole glucose per g cells per day was used to maintain the microbial mass. Under these conditions, this amounts to a maintenance requirement of 9.6 moles ATP per day (3.9 x 0.00624 x 403). Therefore, 39.8 moles of ATP (49.4 - 9.6) are available for bacterial growth which gives a growth yield of 10.1 g cells/mole ATP above maintenance.

APPENDIX C

LABORATORY PROCEDURES

TABLE LIII

POLYETHYLENE GLYCOL (PEG) ASSAY

Reference: Knight, W. M. 1971. An evaluation of intraruminal urea urea infusion for growing lambs. Ph.D. Thesis, University of Illinois, Urbana.

1. Reagents

   a. $BaCl_2$ - $Ba(OH)_2$ Solution.

      Dissolve 96 g of $Ba(OH)_2 \cdot 8\ H_2O$ in approximately 1 liter of glass distilled water (shake until dissolved). Allow to stand 2 days before filtering (Whatman No. 1) and making up to 2 liters. Dissolve 85 g of $BaCl_2$ in glass distilled water and make up to 1 liter. These two solutions are mixed together (3 liter volume) and stored as such. Protect from atmospheric CO_2.

   b. Zinc Sulfate Solution.

      Dissolve 89 g of $ZnSO_4 \cdot 7\ H_2O$ in glass distilled water and make up to 1 liter.

   c. Trichloroacetic Acid (TCA) and Barium Chloride Solution.

      Dissolve 300 g of TCA and 50 g $BaCl_2$ in glass distilled water and make up to 1 liter.

   d. PEG Stock Solution.

      Accurately weigh approximately 4 g of PEG, transfer to a 1 liter volumetric flask and bring to volume with glass distilled water.

TABLE LIII (Continued)

2. Procedure

   a. Centrifuge rumen fluid (not treated with mercuric chloride) at 12,000 x g for 15 minutes.

   b. Pipette 1 ml of sample (supernatant) to centrifuge tube.

   c. Dilute with 8 ml of glass distilled water.

   d. Add 2 ml of $ZnSO_4$ solution.

   e. Add 3 ml of $BaCl_2$ - $Ba(OH)_2$ solution.

   f. Mix thoroughly.

   g. Centrifuge at 2000 x g for 10 minutes to remove precipitate. If supernatant is not clear, filter through Whatman No. 1 filter paper.

   h. Transfer 4 ml of supernatant to test tubes (in duplicate).

   i. Add 4 ml of TCA solution and mix.

   j. Wait 30 minutes before reading against reagent blank at 525 nm in a spectrophotometer. Reading should be fairly stable.

   k. Prepare with each assay standards of 0.2, 0.5, 1.0, 1.5 and 2.0 mg PEG/ml from stock PEG solution. Assay standards according to steps b through j.

TABLE LIV

DETERMINATION OF THE SPECIFIC ACTIVITY OF ^{14}C-LABELED BLOOD GLUCOSE BY THE GLUCOSE PENTAACETATE METHOD

Reference: Jones, G. B. 1965. Determination of the specific activity of labeled blood glucose by liquid scintillation using glucose pentaacetate. Anal. Biochem. 12:249.

Reagents

1. Heparin Solution.

   Dissolve 1 g of sodium heparin (150 U/mg, Nutritional Biochemicals Corporation) in glass distilled water and make up to 1 liter.

2. Barium Hydroxide and Zinc Sulfate Solutions.

   a. $Ba(OH)_2$.

      Dissolve 45 g of $Ba(OH)_2 \cdot 8\ H_2O$ in glass distilled water and make to 1 liter volume. Let stand for at least 1 day. Filter if necessary. Protect from atmospheric CO_2.

   b. $ZnSO_4$.

      Dissolve 50 g $ZnSO_4 \cdot 7\ H_2O$ in glass distilled water and make to 1 liter.

   c. The $Ba(OH)_2$ and $ZnSO_4$ solutions should neutralize each other. To test, add 50 ml of glass distilled water to a flask containing 10 ml of the $ZnSO_4$ solution. Add 2 drops of phenolphthalein (1% solution in 1:1 ethanol:water) and titrate with $Ba(OH)_2$ to a faint pink. It should take 10.0 ± 0.05 ml. If necessary, dilute the more concentrated solution until neutralization is achieved.

3. Glucostat Reagent.

   Dissolve contents of vial (Glucostat x 4) in glass distilled water and make up to 100 ml.

4. Glucostat Chromogen.

   Dissolve contents of chromogen vial in glass distilled water and bring to 100 ml volume.

5. Stock Glucose Solution.

   a. Prepare saturated benzoic acid solution by adding 3.4 g benzoic acid to 1 liter of glass distilled water. Mix

TABLE LIV (Continued)

thoroughly and filter.

b. Dissolve 2.5 g D-glucose (anhydrous) in saturated benzoic acid solution and make up to 1 liter.

c. Let solution stand for several hours to allow mutarotation to reach equilibrium.

6. Saturated GPA Solution.

a. Prepare quantitative amounts of GPA according to Procedure, Step 4 by a larger scale acetylation.

b. Add 470 mg GPA to 500 ml saturated benzoic acid solution. Not all of the GPA will go into solution.

7. ^{14}C-Glucose Solution (0.5 µCi/ml).

a. Dilute 50 µCi of 1-^{14}C-D-glucose in 100 ml of saturated benzoic acid solution.

b. Count (in triplicate) 500 µl in 10 ml of Brays scintillation solvent. Calculate exact concentration of radioactivity per ml. Assume all radioactivity is in glucose.

8. Jones Scintillation Solvent.

Dissolve 5.00 g PPO (2,5-diphenyloxazole) and 0.50 g M_2-POPOP (1,4-bis-2-[4-methyl-5-phenyloxazoyl]-benzene) in toluene and make up to 1 liter volume.

Procedure

1. Prepare blood plasma for storage.

a. Withdraw 50 ml whole blood from animal through jugular catheter via syringe containing 5 ml of heparin solution. Shake gently as blood is being drawn.

b. Immediately transfer heparinized blood to a centrifuge tube and spin at 12,000 x g for 10 minutes at 5°C.

c. Pour plasma into plastic bottles and freeze (-25°C).

d. Discard cells.

2. Prepare (in duplicate) deproteinized blood filtrate (1:20 Somogyi).

a. Thaw blood plasma in refrigerator (4°C).

TABLE LIV (Continued)

b. Transfer 1.5 ml plasma to a 50 ml centrifuge tube.

c. Add 13.5 ml glass distilled water, mix.

d. Add 7.5 ml $Ba(OH)_2$ solution, mix.

e. Add 7.5 ml $ZnSO_4$ solution, mix.

f. Centrifuge at 9800 x g for 10 min at 5°C.

g. Save supernatant and label as protein-free filtrate.

h. Discard pellet.

3. Determine (in duplicate) the glucose content of protein-free filtrate (Glucostat Procedure).

a. Prepare working standards from stock glucose solution as follows:

Standard Conc. (mg/1)	250	500	1000
ml Stock Solution	1.0	2.0	4.0
ml Glass Distilled Water	9.0	8.0	6.0

b. Deproteinize working standards, water blank and samples (usually 10 protein-free filtrate samples plus 3 standards, [in duplicate] = 26 tubes).

c. To a small glass test tube add 2 ml of protein-free filtrate.

d. Add 1 ml of glucostat chromogen, mix.

e. At timed intervals, add 1 ml of glucostat reagent to each tube and mix. Let stand for exactly 10 minutes.

f. At same timed interval, add 1 drop of 4 N HCl and mix.

g. Let stand for at least 5 min; color that is developed is stable for several hours.

h. Read absorbance at 420 nm with spectrophotometer. Set slip width so that reagent blank gives zero absorbance.

i. Find sample blood glucose concentration from standard curve. Multiply by 1.1 to correct for dilution by heparin solution.

4. Prepare (in duplicate) pentaacetate derivative of glucose.

TABLE LIV (Continued)

a. To a 30 ml lipless beaker add 100 mg (weigh exactly) of anhydrous D-glucose.

b. Add 2 drops of glacial acetic acid.

c. Add 20 ml of protein-free filtrate.

d. Evaporate to a thick syrup on a steam bath, assisted by a jet of dry nitrogen.

e. When evaporation is complete, add 60-70 mg (approx.) anhydrous sodium acetate.

f. Add 1 ml of acetic anhydride.

g. Cover beaker with watch glass and place in drying oven (105-110°C) for 1.5 hours.

h. Allow beaker to cool, then add 10 ml of glass distilled water.

i. Boil solution (keep covered) for about 2 min or until all oily globules have disappeared (hydrolysis of remaining acetic anhydride).

j. Allow beaker to cool on bench, stirring occasionally with a glass rod.

k. Place beaker on ice bath for 30 min, stirring occasionally. GPA crystals should form. If necessary, scratch side of beaker slightly.

l. Filter through Buchner funnel containing disk of Whatman No. 4 filter paper.

m. Collect filtrate in Buchner flask, rinse remaining crystals from beaker with filtrate.

n. Rinse beaker and crystals (twice) with small portions (2-3 ml) of saturated GPA solution. Discard filtrate.

o. Transfer crystals from the filter paper back to the beaker, add 10 ml glass distilled water and heat to boiling. Dissolve all crystals.

p. Allow solution to cool, recrystallize and filter as previously described.

q. Transfer GPA crystals to a vial and dry for at least 2 hours at 105°C.

TABLE LIV (Continued)

r. Allow vials to cool in desiccator.

s. Accurately weigh about 100 mg (usually entire yield) of GPA and transfer to a counting vial.

t. Add 10 ml of Jones scintillation fluid and count in a liquid scintillation spectrometer.

u. Correct for quenching by automatic external standardization as determined from quenched standards.

v. Calculate specific activity of blood glucose as:

$$a_g = \left[\frac{G_c + G_s}{G_s} \right] \times 2.1663 \times a_{gpa}$$

where a_g = specific activity of plasma glucose (DPM/mg),

G_c = amount of carrier glucose added (mg),

G_s = amount of glucose present in 20 ml of protein-free filtrate (mg),

a_{gpa} = specific activity of GPA (DPM/mg).

5. Preparation of standard labeled ^{14}C-GPA.

a. To a 30 ml lipless beaker add 0.1 ml of the ^{14}C-glucose solution.

b. Proceed as outlined in Procedure, Step 4 except use protein-free filtrate from an animal which has not been exposed to radioactive substances.

APPENDIX D

DERIVATION OF EQUATION 4.8: CALCULATION OF PLASMA GLUCOSE SPECIFIC ACTIVITY

The total glucose in the reaction beaker can be expressed as

$$G_T = G_c + G_s \tag{D.1}$$

where G_T represents the total amount of glucose, G_c is the amount of 'cold' carrier glucose added and G_s is the amount of glucose in 20 ml of protein-free filtrate. The total amount of glucose radioactivity in the beaker (assumed to come only from G_s) can be expressed as R_T.

To describe the mass relationship of GPA with glucose, consider the reaction

$$\alpha\text{- or } \beta\text{-Glucose} + Ac_2O + NaOAc \longrightarrow \beta\text{-Glucose pentaacetate} \tag{D.2}$$

where Ac_2O represents acetic anhydride and NaOAc is sodium acetate and

$$1 \text{ mg Glucose} \longrightarrow 2.1663 \text{ mg GPA.} \tag{D.3}$$

Therefore, the maximum theoretical amount of GPA that could be formed is

$$GPA_T = 2.1663\ G_T\ . \tag{D.4}$$

The specific activity of the GPA is defined as

$$a_{gpa} = \frac{R_T}{GPA_T} = \frac{R'_T}{GPA'_T} \qquad (D.5)$$

where R'_T and GPA'_T represent the respective quantities of radioactivity and GPA recovered in the chemical analysis. The relationship in Equation D.5 should be valid since any loss of glucose radioactivity will be accompanied by a similar reduction in the amount of GPA produced. This is based on the assumption that R_T is evenly distributed throughout G_T and any yield of Reaction D.2 less than 100% will not affect the specific activity because both R_T and GPA_T will be reduced by a common factor, f, where $0 < f \leq 1$.

Similarly,

$$a_{gb} = \frac{R_T}{G_T} = \frac{R'_T}{G'_T} \qquad (D.6)$$

where a_{gb} is the specific activity of the glucose in the beaker. Equation D.4 (corrected for 100% yield) into Equation D.6 gives

$$a_{gb} = 2.1663 \frac{R'_T}{GPA'_T} = 2.1663 \cdot a_{gpa} . \qquad (D.7)$$

The specific activity of the glucose in the protein-free filtrate (plasma glucose) is

$$a_g = \frac{R_T}{G_s} = \frac{R'_T}{G'_s} . \qquad (D.8)$$

Equating R'_T in Equation D.6 and D.8 gives

$$a_{gb} \cdot G'_T = a_g \cdot G'_s . \qquad (D.9)$$

Substituting for a_{gb} from Equation D.7 and rearranging yields

$$a_g = 2.1663 \cdot a_{gpa} \cdot \frac{G'_T}{G'_s} \tag{D.10}$$

but,

$$\frac{G'_T}{G'_s} = \frac{f \cdot G_T}{f \cdot G_s} = \frac{G_T}{G_s} \tag{D.11}$$

so, using Equation D.1,

$$a_g = 2.1663 \cdot a_{gpa} \cdot \left[\frac{G_c + G_s}{G_s} \right] . \tag{D.12}$$

APPENDIX E

RUMEN VFA AND RUMEN FLUID (48,000 x g)

SPECIFIC ACTIVITY DATA

This appendix contains the VFA specific activity data for each infusion experiment. These data are not corrected for differences in infusion rate, steer weight or feed rate. In addition, the specific activity of the 48,000 x g supernatant is presented.

TABLE LV

RUMEN FLUID AND VFA SPECIFIC ACTIVITY FOR ANIMAL 608 CONSUMING THE WHOLE CORN DIET[a]

Expt.	Time[b] (hr)	Specific Activity (μCi/gac)[c]					a_s[d] (nCi/ml)
		a_a	a_p	a_b	a_{iv}	a_v	
Ac-WC	1.03	27.61	0.689	6.35	-	-	5.69
Ac-WC	2.11	27.90	1.298	10.52	-	-	5.87
Ac-WC	3.11	28.16	1.591	11.78	-	-	6.46
Ac-WC	4.06	27.43	1.953	13.49	-	-	6.11
Ac-WC	5.11	27.26	2.054	12.64	6.08	9.80	6.41
Ac-WC	6.11	27.35	2.000	12.51	5.69	9.95	6.68
Ac-WC	7.11	24.51	1.852	12.82	5.71	7.82	6.47
Ac-WC	8.16	11.78	1.617	9.46	4.41	6.71	3.61
Ac-WC	9.06	7.06	1.417	7.43	4.06	5.88	2.71
Pr-WC	1.00	0.841	46.70	0.178	-	-	4.10
Pr-WC	2.00	0.914	48.45	0.205	-	-	4.24
Pr-WC	3.00	1.144	54.72	0.258	-	-	4.44
Pr-WC	4.00	1.065	47.18	0.285	-	-	3.94
Pr-WC	5.00	1.070	46.25	0.323	15.09	13.72	3.88
Pr-WC	6.00	1.060	46.61	0.335	16.14	12.73	3.78
Pr-WC	7.00	0.873	29.62	0.331	13.71	11.07	2.86
Pr-WC	7.92	0.572	18.24	0.267	10.39	8.68	1.73
Pr-WC	9.00	0.282	9.29	0.202	5.48	6.21	1.07
Bu-WC	1.00	1.609	0.331	32.17	-	-	2.39
Bu-WC	2.00	1.970	0.368	24.22	-	-	1.84
Bu-WC	3.00	2.190	0.420	22.01	-	-	1.82
Bu-WC	4.00	2.255	0.552	22.58	-	-	1.91
Bu-WC	5.00	2.259	0.685	22.91	-	-	1.90
Bu-WC	6.00	2.242	0.676	21.78	-	-	1.96
Bu-WC	6.50	2.257	0.601	21.17	-	-	1.80
Bu-WC	7.00	2.089	0.506	16.57	-	-	1.49
Bu-WC	7.50	1.748	0.464	13.45	-	-	1.31
Bu-WC	8.00	1.526	0.408	10.11	-	-	1.18
Bu-WC	8.50	1.199	0.384	7.20	-	-	0.81
Bu-WC	9.00	0.981	0.366	5.16	-	-	0.72

[a]Data is not corrected for infusion rate, steer rate or feed rate.

[b]Time is relative to injection of priming dose.

[c]a- acetate, p- propionate, b- butyrate, iv- isovalerate fraction (2MB + 3MB), v- valerate.

[d]Specific activity of 48,000 x g supernatant.

TABLE LVI

RUMEN FLUID AND VFA SPECIFIC ACTIVITY FOR ANIMAL 422 CONSUMING THE WHOLE CORN DIET[a]

Expt.	Time[b] (hr)	Specific Activity (μCi/gac)[c]					a_s[d] (nCi/ml)
		a_a	a_p	a_b	a_{iv}	a_v	
Ac-WC	1.00	49.62	0.559	7.51	-	-	6.01
Ac-WC	2.00	42.52	0.964	11.76	-	-	6.35
Ac-WC	3.17	35.78	1.374	16.97	-	-	8.84
Ac-WC	4.00	31.81	1.423	16.11	-	-	7.94
Ac-WC	5.00	31.16	1.426	16.99	11.25	10.13	8.72
Ac-WC	6.00	31.67	1.416	17.40	11.38	10.37	7.58
Ac-WC	7.00	22.44	1.369	14.14	10.91	8.99	7.95
Ac-WC	8.00	13.90	1.268	12.67	9.36	7.52	6.17
Ac-WC	9.00	7.74	1.091	10.25	7.68	6.47	4.28
Pr-WC	1.00	0.484	12.02	0.136	-	-	1.89
Pr-WC	2.00	0.671	21.15	0.491	-	-	3.69
Pr-WC	3.00	0.825	32.42	0.819	-	-	6.54
Pr-WC	4.00	0.794	31.47	0.900	-	-	6.39
Pr-WC	5.00	0.799	32.02	0.938	6.85	8.37	7.54
Pr-WC	6.00	0.801	31.61	0.937	6.59	8.34	6.52
Pr-WC	7.00	0.725	26.23	0.890	6.38	7.06	6.79
Pr-WC	8.00	0.515	21.83	0.888	5.37	5.99	5.87
Pr-WC	9.00	0.351	18.33	0.768	4.81	6.03	5.32
Bu-WC	1.00	0.723	0.121	10.08	-	-	1.34
Bu-WC	2.00	2.013	0.220	18.91	-	-	2.63
Bu-WC	3.00	2.148	0.212	17.70	-	-	2.69
Bu-WC	4.00	2.120	0.211	16.03	-	-	2.92
Bu-WC	5.00	2.141	0.211	15.22	-	-	2.93
Bu-WC	6.00	2.118	0.218	15.95	-	-	3.11
Bu-WC	6.92	1.991	0.175	13.40	-	-	2.81
Bu-WC	7.92	1.651	0.138	10.59	-	-	2.48
Bu-WC	9.00	1.211	0.117	9.23	-	-	2.08

[a]Data is not corrected for infusion rate, steer rate or feed rate.

[b]Time is relative to injection of priming dose.

[c]a- acetate, p- propionate, b- butyrate, iv- isovalerate fraction (2MB + 3MB), v- valerate.

[d]Specific activity of 48,000 x g supernatant.

TABLE LVII

RUMEN FLUID AND VFA SPECIFIC ACTIVITY FOR ANIMAL 612 CONSUMING THE GROUND CORN DIET[a]

Expt.	Time[b] (hr)	Specific Activity (μCi/gac)[c]					a_s[d] (nCi/ml)
		a_a	a_p	a_b	a_{iv}	a_v	
Ac-GC	1.00	60.32	0.906	24.60	-	-	8.23
Ac-GC	2.10	65.50	1.530	38.54	-	-	10.78
Ac-GC	3.10	74.19	1.997	43.84	-	-	11.85
Ac-GC	4.10	74.78	2.246	52.38	-	-	12.98
Ac-GC	5.10	75.63	3.010	54.06	13.19	27.11	12.93
Ac-GC	6.10	75.40	3.849	54.29	14.39	27.24	12.73
Ac-GC	7.10	38.27	3.610	48.82	13.23	25.37	9.49
Ac-GC	8.10	23.21	3.132	42.80	12.30	19.33	6.96
Ac-GC	9.10	15.34	2.584	33.24	11.28	14.53	5.23
Pr-GC	0.90	1.879	17.64	0.504	-	-	3.35
Pr-GC	2.00	2.238	19.47	1.049	-	-	4.16
Pr-GC	3.00	2.910	23.87	1.918	-	-	5.12
Pr-GC	4.00	2.418	22.65	2.190	-	-	5.31
Pr-GC	5.00	2.012	20.03	2.455	5.37	14.03	5.24
Pr-GC	6.10	2.033	20.61	2.443	5.47	13.77	5.59
Pr-GC	7.00	1.484	15.87	1.944	5.12	11.98	4.84
Pr-GC	8.00	1.005	12.34	1.730	4.28	9.32	4.07
Pr-GC	9.00	0.707	9.55	1.639	3.44	7.41	3.17
Bu-GC	1.10	1.522	0.122	39.11	-	-	2.38
Bu-GC	2.10	2.430	0.188	38.03	-	-	2.53
Bu-GC	3.10	2.754	0.242	35.53	-	-	2.53
Bu-GC	4.10	2.926	0.254	28.99	-	-	2.43
Bu-GC	5.10	2.711	0.261	28.80	-	-	2.43
Bu-GC	6.00	2.887	0.282	29.06	-	-	2.66
Bu-GC	7.00	2.764	0.351	26.92	-	-	2.09
Bu-GC	8.10	2.347	0.337	19.75	-	-	1.57
Bu-GC	9.10	1.730	0.303	12.16	-	-	1.05

[a]Data is not corrected for infusion rate, steer weight or feed rate.

[b]Time is relative to injection of priming dose.

[c]a- acetate, p- propionate, b- butyrate, iv- isovalerate fraction (2MB + 3MB), v- valerate.

[d]Specific activity of 48,000 x g supernatant.

TABLE LVIII

RUMEN FLUID AND VFA SPECIFIC ACTIVITY FOR ANIMAL 608 CONSUMING THE GROUND CORN DIET[a]

Expt.	Time[b] (hr)	Specific Activity (μCi/gac)[c]					a_s[d] (nCi/ml)
		a_a	a_p	a_b	a_{iv}	a_v	
Ac-GC	1.20	177.10	2.150	36.34	-	-	16.3
Ac-GC	2.20	450.10	3.667	49.54	-	-	38.9
Ac-GC	3.20	101.80	4.228	46.41	-	-	11.7
Ac-GC	4.20	72.39	4.660	51.53	-	-	11.3
Ac-GC	5.20	52.86	3.910	52.63	17.24	31.17	11.6
Ac-GC	6.20	52.98	3.514	51.31	16.87	31.72	12.0
Ac-GC	7.10	41.05	3.577	50.32	9.53	25.51	9.58
Ac-GC	8.10	25.19	3.037	41.19	7.90	22.19	7.77
Ac-GC	9.00	15.29	2.319	28.48	6.94	16.84	6.08
Pr-GC	1.10	1.995	7.75	0.158	-	-	1.39
Pr-GC	2.20	4.285	17.73	0.814	-	-	3.83
Pr-GC	3.00	6.103	28.90	0.593	-	-	6.50
Pr-GC	4.00	6.629	30.91	1.419	-	-	7.10
Pr-GC	4.90	6.402	31.59	1.487	8.15	18.96	7.33
Pr-GC	6.00	6.503	30.82	1.494	8.20	18.64	8.13
Pr-GC	7.00	0.992	20.04	1.272	7.88	16.94	5.35
Pr-GC	8.00	0.832	14.94	1.043	4.88	11.31	3.79
Pr-GC	9.00	0.574	10.16	0.897	4.13	9.31	2.44
Bu-GC	2.00	4.565	1.331	74.97	-	-	9.46
Bu-GC	3.10	6.367	1.939	47.38	-	-	7.32
Bu-GC	4.00	4.080	1.088	52.50	-	-	8.22
Bu-GC	5.00	3.888	1.094	53.08	-	-	8.59
Bu-GC	6.00	3.971	1.116	52.84	-	-	9.06
Bu-GC	7.00	2.135	0.338	21.69	-	-	4.49
Bu-GC	7.90	1.499	0.214	13.81	-	-	2.98
Bu-GC	8.90	1.349	0.157	6.35	-	-	1.65

[a]Data is not corrected for infusion rate, steer weight or feed rate.

[b]Time is relative to injection of priming dose.

[c]a- acetate, p- propionate, b- butyrate, iv- isovalerate fraction (2MB + 3MB), v- valerate.

[d]Specific activity of 48,000 x g supernatant.

APPENDIX F

TURNOVER RATE CONSTANTS FOR EACH VFA MODEL

This appendix lists the turnover rate constants for the complete three compartment model and the incomplete four compartment model.

TABLE LIX

TURNOVER RATE CONSTANTS AND THEIR ASSOCIATED ERROR FOR THE COMPLETE THREE COMPARTMENT VFA MODEL[a,b]

Parameter[c] / Period[d]	Term[e]	k_{aa}	k_{oa}	k_{ap}	k_{ab}	k_{pp}	k_{op}	k_{pa}	k_{pb}	k_{bb}	k_{ob}	k_{ba}	k_{bp}
608-WC	m	8.595	4.644	0.3629	2.110	11.55	11.24	0.3025	0.4233	15.53	13.00	3.649	-0.05345
	e	0.3820	0.4364	0.008393	0.06580	1.071	1.071	0.01858	0.01370	0.9994	1.002	0.2102	0.01017
422-WC	m	15.64	11.63	0.1774	1.698	5.767	5.513	0.5115	0.05401	6.725	4.973	3.496	0.07638
	e	3.100	3.292	0.03246	0.2873	0.9224	0.9230	0.1352	0.005808	0.6684	0.7276	0.7629	0.001710
612-GC	m	8.490	4.155	0.4944	1.873	4.991	4.485	0.3146	0.1421	0.716	7.701	4.020	0.01211
	e	0.9062	1.009	0.06261	0.2315	0.8081	0.8108	0.01694	0.02177	1.121	1.145	0.4434	0.02012
608-GC	m	16.66	14.42	1.927	1.643	4.606	2.829	0.6659	-0.008522	2.783	1.149	1.571	-0.1500
	e	3.185	3.192	0.3487	0.3877	0.7536	0.8305	0.1195	0.005458	0.3134	0.4986	0.1730	0.01401
608-GC[f]	m	17.94	11.63	2.049	3.917	4.602	3.095	0.6299	0.05520	10.06	6.088	5.682	-0.5423
	e	3.424	3.487	0.3696	0.8175	0.7536	1.060	0.1187	0.003810	1.171	1.428	0.6474	0.05275
WC	m	11.01	7.500	0.2350	1.654	7.692	7.396	0.3789	0.1453	9.323	7.524	3.131	0.06144
	e	3.552	4.052	0.09829	1.174	2.571	2.573	0.1415	0.1024	3.972	4.143	1.944	0.04206
GC	m	11.00	8.555	0.9828	1.393	4.779	3.895	0.4503	0.03534	4.303	2.875	1.995	-0.09872
	e	4.033	4.277	0.07164	0.9847	0.2216	0.2394	0.1583	0.01459	2.456	2.646	1.414	0.05561

[a] Values were calculated by solving Equation (2.54) using the data in Tables XLI and XLII.

[b] Values are expressed in units of $min^{-1} \times 10^{-3}$.

[c] k_{ij} represents the rate constant associated with the flow of carbon from compartment j to compartment i.

[d] Animal-ration period or diet.

[e] m - rate constant; e - error term.

[f] Total radioactivity data for infusion 608-Bu-GC was replaced by that obtained during the 612-Bu-GC infusion experiment.

TABLE LX

TURNOVER RATE CONSTANTS FOR THE INCOMPLETE FOUR COMPARTMENT VFA MODEL[a,b]

Parameter[c] / Period[d]	k_{aa}	k_{oa}	k_{ap}	k_{ab}	k_{pp}	k_{op}	k_{pa}	k_{bb}	k_{ob}	k_{ba}	k_{vv}	k_{va}	k_{vp}
608-WC	8.595	4.421	0.3629	2.110	11.54	11.02	0.4310	15.53	13.42	3.646	3.632	0.09692	0.1599
422-WC	15.64	11.39	0.1774	1.698	5.766	5.510	0.5677	6.724	5.026	3.508	2.718	0.1767	0.07834
612-GC	8.490	3.859	0.4944	1.873	4.991	4.315	0.3943	9.716	7.843	4.022	4.324	0.2147	0.1814
608-GC	16.66	14.36	1.927	1.643	4.603	2.554	0.6399	2.761	1.118	1.358	4.303	0.2975	0.1219
608-GC	17.94	12.16	2.049	3.917	4.608	2.437	0.6902	9.784	5.867	4.779	4.303	0.3118	0.1222
WC	11.01	7.294	0.2350	1.654	7.689	7.348	0.4556	9.322	7.668	3.136	3.175	0.1245	0.1058
GC	11.00	8.319	0.9828	1.393	4.782	3.655	0.4916	4.296	2.903	1.940	4.314	0.2490	0.1439

[a] Values were calculated by solving Equations (2.44) and (2.46) using the data in Tables XXXIX and XL assuming k_{pb}, k_{bp}, k_{av}, k_{pv}, k_{bv}, $k_{vb} = 0$.

[b] Values are expressed in units of $min^{-1} \times 10^{-3}$.

[c] k_{ij} represents the rate constant associated with the flow of carbon from compartment j to compartment i.

[d] Animal-ration period or diet.

VITA

William Michael Sharp

Candidate for the Degree of

Doctor of Philosophy

Thesis: A COMPARTMENTAL ANALYSIS OF THE PRODUCTION, INTERCONVERSION AND METABOLISM OF VOLATILE FATTY ACIDS BY THE RUMEN MICROBIAL ECOSYSTEM IN STEERS CONSUMING A WHOLE OR GROUND CORN HIGH-CONCENTRATE DIET

Major Field: Animal Nutrition and Biochemistry

Biographical:

Personal Data: Born in Nowata, Oklahoma, October 30, 1948, the son of William L. and Patricia L. Sharp.

Education: Graduated from John Marshall High School, Oklahoma City, Oklahoma, in 1966. Received the Bachelor of Science degree from Oklahoma State University with a major in Animal Science--Science option in May, 1970. Completed the requirements for the Doctor of Philosophy in Animal Nutrition and Biochemistry at Oklahoma State University in July, 1977.

Professional Experience: Raised and worked on a livestock and grain farm in Northeastern Oklahoma; Graduate Research and Teaching Assistant in the Department of Animal Science and Industry, Oklahoma State University, Stillwater, Oklahoma, 1971-1976.

Professional Organizations: Member of American Society of Animal Science, American Dairy Science Association, Phi Kappa Phi.