INVESTIGATION OF SOME FACTORS AFFECTING VOLATILE FATTY ACID TRANSFER ACROSS RUMEN EPITHELIUM IN AN IN VITRO SYSTEM

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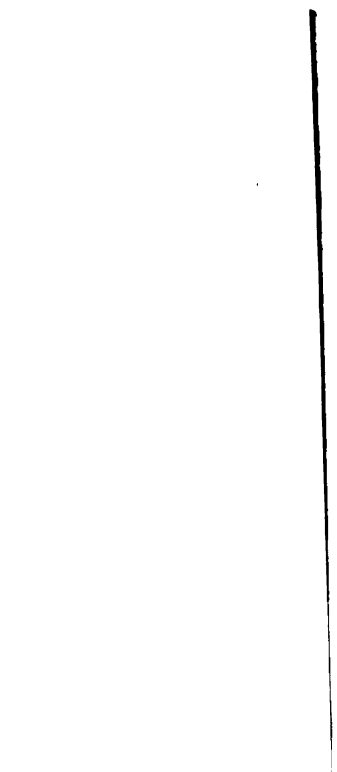

ABSTRACT

INVESTIGATION OF SOME FACTORS AFFECTING VOLATILE FATTY ACID TRANSFER ACROSS RUMEN EPITHELIUM IN AN *IN VITRO* SYSTEM

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An *in vitro* system for studying transfer across rumen epithelium is described and results of changing parameters are given. Although the constant maximum rate of volatile fatty acid transfer was not measured, due to inclusion of the lag time, this lag time of serosal appearance was reduced by higher fatty acid levels and by decreased pH, and was independent of fatty acid chain length. Among fatty acids, serosal accumulation at two hours is a measure of relative transfer rate.

Parameters investigated and results included:

1) Mucosal concentration and fatty acid chain length on serosal accumulation. Sample regression coefficients were:

Acetate: $y = 0.301 X - 0.179$

Propionate: $y = 0.454 X - 0.250$

Butyrate: $y = 0.166 X - 0.048$

There was a significant departure from linear regression of serosal accumulation on mucosal concentration of acetate and propionate.

2) Decrease in pH to 4.0 increased ($P < 0.01$) serosal accumulation of acetate and propionate. HCl caused a further increase ($P < 0.01$) in acetate accumulation relative to H_2SO_4 as the adjusting medium.

3) Serosal glucose and mucosal HCN did not affect acetate accumulation and serosal butyrate reduced acetate accumulation in one instance. Both serosal glucose and serosal butyrate reduced propionate accumulation at some concentrations.

4) Back transfer moved a larger percentage of C^{14}-butyrate carbon than mucosal-to-serosal transfer of unlabeled butyrate. The amount of back transfer was not enough to affect other results.

5) There was less serosal accumulation with biopsy tissue than with slaughter tissue.

These results are consistent with the thesis that volatile fatty acid transfer is a passive process, modified by epithelial metabolism.

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By

John Walter Bell

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DEDICATION

This volume is respectfully dedicated to my parents and teachers who taught by example that worthwhile things must be sought diligently.

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INTRODUCTION

The ruminant's ability to increase the nutritional value of carbohydrate and protein sources for the human population is both historic fact and future promise. The studies of the past one hundred years have defined many of the processes by which the rumen microbiota accomplish this task. As knowledge accumulates concerning the amounts and variety of materials leaving the rumen through the rumen epithelia, its importance as an agent between the microbiota and host becomes apparent. Further study of epithelial contribution and function is necessary and no method of studying this tissue is completely satisfactory under all conditions. A method of study less dependent upon host and microbiota variation would be useful for many purposes and further adaptation of *in vitro* methods approaches this goal.

This project was initiated with three purposes:

1) The development of a simple *in vitro* system for studying transfer across the rumen epithelium.

2) The comparison of responses of this system with responses of *in vivo* and other *in vitro* methods to such experimental parameters as might be imposed.

3) The examination of the processes by which materials are transferred across the rumen epithelium.

REVIEW OF LITERATURE

In many respects the rumen may be considered as a pool. This is especially so when studying absorption from the rumen. The stream enters through the esophagus and exits via the omasal orifice. In addition, materials enter as secretions and other exits exist as absorption through the rumen wall or eructed gasses. Interactions between the pool inlets and outlets cause the volume of the rumen pool to constantly change. The pool composition is also constantly changing, due to internal production and consumption of metabolites and possibly differential absorption.

This review of literature is divided into three sections:

1. Methods of evaluating passage of materials into and out of the rumen, including *in vivo*, perfused rumen, and *in vitro* methods.

2. Factors affecting the rate of disappearance of volatile fatty acids from the rumen.

3. Factors affecting appearance of volatile fatty acids in portal blood.

Methods of Studying Rumen Transfer: General Considerations

Pool Size and Heterogeneity

Under some circumstances the problems of variation in volume and the heterogeneity of the contents may be studied concurrently. Rumen volume may be determined by removing the rumen contents and measuring the volume and rumen composition by mixing and sampling while the contents are removed. The materials may then be returned to the rumen or a material of known composition substituted. The disadvantages of these methods include disruption of rumen fermentation, restriction of studies to animals with rumen fistulas, and the impossibility of performing rapid time sequence experiments because of the volume of materials.

Marker techniques afford a second means of determining rumen volume, a means whose validity is dependent upon the uniformity with which the marker is distributed

throughout the rumen. Under natural conditions this distribution is sometimes incomplete because of time limits and natural layering of rumen contents. Pumps may be used to mix the materials more rapidly, but are largely ineffective when long hay or similar roughage is present. Johnson (28) included a discussion of available marker materials and analytical techniques in his review of rumen study procedures.

Inlets to the Rumen

1) Esophagus. The principle avenue of material inflow to the rumen is the esophagus, which carries both food and saliva. Both amount and content of food may be controlled. The common method is to withhold food entirely, or if some intake is essential, the animal may be trained to consume all of a known amount within a specified time limit. As long as the food is relatively homogeneous, animals will not discriminate against any portion. However, if the ration contains long roughage as well as concentrate, the animal may refuse part of the roughage or the smaller particles may separate by gravity

and knowledge of composition as well as amount of refused feed is necessary and not always accurate.

Saliva, as a secretion of the animal, is not easily controlled. Various stimuli have been shown to affect not only the amounts of saliva secreted, but also its composition. Saliva contains large amounts of sodium, potassium, and magnesium bicarbonates. Ordinarily only the sodium and magnesium ions are of interest, because of the large amounts of potassium in ruminant rations. With increasing interest in non-protein-nitrogen sources and their metabolism, the urea content of saliva and its pool kinetics are of interest, the mode of entrance being a basic part of these studies. Saliva is an effective buffering agent (55), due in part to the bicarbonates, and many rumen reactions are influenced by pH value.

Because of these considerations, there are three choices: 1) Ignore the contribution of the saliva, 2) drain off the saliva, or 3) drain, sample, and measure the saliva, and return it to the rumen. If course two or three is followed, and saliva is to be drained, the techniques available include the esophageal cannula (28) and the esophageal catheter (18, 50). The former may be

forced out of the fistula, the latter adds stress at the time of the experiment by the stimulation of the cardia during the experiment. With these methods, saliva volume and constituents may be determined and the saliva returned to the animal. If the saliva is not returned to the animal, it must be replaced by a solution of equivalent buffering capacity because of the sensitivity of rumen fermentation to pH value changes.

2) The Rumen Epithelium. The materials passing through the epithelium into the rumen may be divided into three classes: organic, inorganic, and water. Water passage into the rumen resulting from differences in osmotic pressure may play a role in controlling the concentrations of materials in the reticulorumen. Murray et al. (35) suggest that changes in osmotic pressure and water passage influence the amounts of rumen contents and influence the outflow of rumen fluid to the omasum. Warner and Stacy (60) noted that post-prandial increases in rumen content osmotic pressure was accompanied by signs of haemoconcentration, indicating a transfer of water from the blood into the alimentary tract, including the rumen. Stacy and Brook (44) concluded that the renal response of sheep

to feeding was partly due to water withdrawal from the blood into the rumen. The work of Parthasarathy and Phillipson (36) indicated that, in anaesthetized sheep with a surgically isolated rumen, hypertonic solutions within the rumen gained water as a result of water movement across the rumen wall. This dilution of rumen contents as a result of an increase in the osmotic pressure could serve to obscure increases in concentration if concurrent rumen volumes are not determined.

Annison (1b) reported that blood volatile fatty acids entered the rumen to bring final volatile fatty acid concentrations of originally volatile fatty acid-free solutions close to that of blood. This would probably be true of all small organic molecules.

However, especially in the inorganic classification, not all assumed secretions via the rumen epithelium have been substantiated. Smith et al. (41) measured secretion of isotopic phosphorus into rumen contents, using assumed salivary secretion rates, and reported that the amount of phosphorus transferred was greater than could be accounted for by salivary secretion. Their alternative explanation was that substantial amounts of phosphate must

enter the rumen via its epithelium. However, this would require that phosphate move against both concentration and potential gradients through the rumen epithelium. According to Hyden (26) and Ash and Dobson (7), rumen epithelium is only slightly permeable to the phosphate ion in either direction. With revised secretion rates Annison and Lewis (3b) concluded that endogenous phosphorus in the rumen came from saliva.

These examples serve to illustrate both the difficulty and the importance of being able to measure the contributions of the saliva and rumen epithelium to rumen contents.

Outlets from the Rumen

In the intact animal, there are three outlets from the rumen, the esophagus, reticulo-omasal opening, and the rumen epithelium.

1) The esophagus functions as an outlet for gasses from the rumen. Loss of fermented materials from the rumen as carbon dioxide and methane usually constitute five to seven percent of the gross energy consumed (34). Negative pressure applied to the gas space at the top of

the rumen, via a rumen fistula, with measurement of the amount and concentration of materials in the effluent is one means of measuring these losses.

2) The reticulo-omasal opening is the exit for all solids and part of the liquids leaving the rumen. Methods of dealing with omasal outflow are usually variations of three approaches: 1) Disregard this outflow, 2) Close the opening, assuming that changes in rumen and animal metabolism will not interfere with the experiment, or 3) Use a marker to measure omasal outflow. The choice is made on the essentiality of knowing how much material leaves the rumen via this route, the acuteness of the experiment, and the effect of blocking the reticulo-omasal opening on the metabolism of the animal. The first approach was used by Barcroft et al. (8) in their pioneering studies which demonstrated that the volatile fatty acids were absorbed from the rumen. For the purpose of their investigation, it was only necessary to demonstrate the appearance of volatile fatty acids in the rumen vein. The second method, which they also used, requires ligation of the neck of the omasum and is unusable for many investigations because of the extensive surgery required. Other

disadvantages include the unphysiological state caused by the disturbance of the digestive organs, anesthesia, and the inability to use the animal more than once, preventing inter-treatment comparisons.

The third approach to measuring omasal passage is to replace the rumen contents with a known amount of liquid containing a known amount of a marker. Johnson (28) discussed several of these substances and their limitations as well as references where detailed protocols for their usage may be found. Use of markers for this purpose is predicated upon a strict relationship between the marker and the material under investigation. Ideally as a quantity of material being studied leaves the rumen via the reticulo-omasal opening, it is accompanied by a proportional amount of marker. If, however, there is a departure from strict proportionality caused by a unilateral loss or gain of either study material or marker, errors will be introduced. A restriction to marker use is the absorption onto the wall of the rumen and upon particles of ingesta in the rumen.

Another area of concern is the behavior of the replacement fluid. In most biological solutions water is the solvent. Rumen fluid is no exception, and

consequently, most rumen fluid replacements use water as the solvent. Water, however, has the ability to diffuse across the rumen epithelium in response to osmotic pressure gradients, and experiments which depend on material: marker ratios to evaluate absorption require careful study to detect water flux.

If absorption in the entire fore-stomach is to be considered, the omasal-abomasal fistula described by Kameaka and Morimoto (29) might be considered. A slightly different approach would be the re-entrant duodenal fistula of Conver, McGilliard, and Huffman (16).

3) The rumen epithelium is the most important exit from the rumen in terms of dry matter disappearance. Measuring transfer through the rumen epithelium may be done directly or indirectly.

Direct methods of measuring rumen epithelium transfer involves measurement of arteriovenous differences in concentration and blood flow rate of the material being studied. Measuring rumen absorption by comparison of rumen or portal vein blood concentrations with concentrations in arterial blood is surgically difficult (7). In contrast to the abomasum, the rumen is drained by more than one vein (40). These veins discharge their contents

at various points into the portal vein, which contains blood draining from the more distal portions of the digestive tract. There are two alternatives, both less than satisfactory, measuring less than the complete rumen contribution or including contributions from more distal portions of the digestive tract. A further limitation of measuring arteriovenous differences is that, without a measure of blood flow, no quantification of materials absorbed is possible.

Another disadvantage to the direct method is the selective metabolism of the rumen epithelium. Sutherland (49) cites a number of reports to indicate that rumen epithelium, both in vitro and in vivo, metabolizes butyric acid to ketone bodies, and under natural conditions, little butyrate appears in the portal flow (2). These reports indicated limited metabolism of acetate in the rumen wall, most being carried unchanged to the liver, and limited metabolism of propionic acid to lactic acid.

The indirect approach is described by Sutton et al. (50) using young calves. Absorption was calculated as the amount disappearing from the rumen that could not be accounted for by other exits. In this case the only

exit was the omasal orifice, and disappearance via this route was calculated using polyethylene glycol as a marker.

Isotopic Methods

Another way of studying rumen absorption is the use of isotopes. Isotopes are especially valuable when used in combination with other techniques, which can help to solve the problem of recirculation of materials from the rumen to the blood and back. Discussions of their uses, advantages, and limitations, as well as specific applications, are given by Annison (1a) and Cook (17).

The Rumen Pouch Method

A method to alleviate some of the difficulties encountered in the incompletely blocked off rumen inlets and outlets is described by Tsuda (51, 52). In this method a portion of the rumen is surgically closed to make a blind pouch opening to the outside through a fistula. In measuring the relationship between rumen motility and volatile fatty acid absorption, the amounts

absorbed were expressed as percent of initial concentration (53). Absorption rate was dependent upon dilution and in later work, Tsuda (54) found that the direction of water flux depended upon the concentration of materials in the rumen.

Perfused Rumen Techniques

In further efforts to control rumen inlets and outlets and remove other variables, perfused rumen techniques, such as that described by Brown (13) have been employed. These methods use a rumen excised from the animal, suspended so that material may be placed in it, and perfused with the collected, oxygenated blood of the donor animal. These preparations have many of the same limitations as acute experiments, but do have the advantage of recirculating blood without metabolism, so that slowly absorbed materials may be concentrated. However, the low blood flow rate, lack of liver and kidney function to maintain normal metabolite levels, and gradual anoxia make the perfused rumen a method used primarily for special uses.

In Vitro Methods of Studying Rumen Absorption

The requirements and limitations of *in vivo* methodology have caused some investigators to turn to *in vitro* methods. Early *in vitro* devices for studying intestinal absorption in both ruminants and non-ruminants are described by Wilson (62). A device first used by Ussing and Zerhan (57) to study transport of sodium in frog skin and more recently used by Stevens (45, 46, 47, 48) for the study of rumen epithelial transport is a more elaborate version of these early approaches. Hird and Weidemann (25) described a similar device and used it to study transport and metabolism of butyrate by rumen epithelium. Both of these systems suspend rumen epithelium between two chambers containing fluids representing the blood and rumen contents.

Factors Affecting Volatile Fatty Acid Disappearance from the Rumen

The volatile fatty acids have been shown to be the main source of energy for the ruminant (3a) and the main product of cellulose digestion. In this group of

substances there is a range of properties and conditions that affect their absorption and utilization. In this section the properties and conditions that affect rumen disappearance are discussed.

Influence of Volatile Fatty Acid Chain Length on Their Disappearance from the Rumen

Danielli et al. (19) first reported that volatile fatty acids disappear from an acid medium in the order butyric > propionic > acetic. Gray (22) confirmed that propionate was absorbed more rapidly than acetate. Gray and Pilgrim (24) combined the results of two methods, absorption as measured by disappearance from the rumen, and volatile fatty acid production as measured by in vitro fermentation studies, and suggested that propionate was absorbed from the rumen more rapidly than acetate and butyrate. This conclusion is dependent upon the correspondence of in vivo and in vitro volatile fatty acid production rates. Johnson (27) used goats, and found the order of disappearance to be butyrate > propionate > acetate. These reports indicate that in vivo, the longer the volatile fatty acid chain, the more rapidly it is

absorbed. This would indicate that the size of the molecule *per se* is not the limiting factor in rumen absorption. If that were the case, the smaller molecule, the shorter chain, would be absorbed faster.

Stevens and Stettler (47) compared acetate and butyrate in their *in vitro* preparations and found that butyrate disappeared two to three times as rapidly at both pH 6.4 and 7.4. They later (48) proposed an active transport system for acetate in the blood to lumen direction. This transport was not of such magnitude as to account for the difference in net acetate and butyrate transfer rates.

Because of their common substrates, the volatile fatty acids, comparisons might be made between the rumen epithelium and the intestinal mucosa. A distinction should be made, however, between the dual substrates of the intestinal mucosa where both long and short chain fatty acids are available in the intestinal contents, and the rumen epithelium, because of the paucity of free long chain fatty acids in rumen contents. Bloom *et al*. (12) established the difference between the transfer of short chain fatty acids which appear free in the portal flow,

and the long chain acids ($>^{C}10$) which appear mainly in the lymph in the form of triglycerides. Senior (39) wrote one review of work in the latter field. Absorption of the volatile fatty acids is thought to involve a combination of passive penetration and a fatty acid permease in the intestine. Micelle formation, proposed as a limiting factor in the decreasing capacity of fatty acids to be absorbed at higher chain lengths by Carr, as quoted by Coe (14), would be limited with the short chain fatty acids and passive penetration would be determined either by diffusion through aqueous channels or by transfer across lipid membranes.

Benzene:water partition coefficients are used as criteria of lipid:water solubility. The benzene:water partition coefficients would predict that passive penetration rates through lipid membranes should increase nearly three hundred times with an increase in chain length from two to six carbon atoms. Barry and Smyth (9) observed only a 20 percent increase in rate of absorption <u>in vivo</u> as chain length increased from two to six carbon atoms. This would imply that passive transfer via lipid membranes is of minor importance for acids

below C_6. If passive transport across lipid membranes may be based on the simple oil:water partition coefficients, the failure to find increases in transfer corresponding to the increases in oil:water partition coefficients indicates that (1) active transfer tends to increase with decreasing chain length, or (2) passive transfer by aqueous pathways is a major factor.

In a report by Coe and Coe (15) and expanded by Coe (14) the hypothesis is advanced that the vapor pressure of the solute may be an important factor in the rate of penetration through biological membranes. According to their equations, passive transfer rate through a lipid membrane could actually decrease with increasing chain length. On this basis, it would be difficult to distinguish between aqueous and lipid pathways.

Effect of Volatile Fatty Acid Concentration on Disappearance from the Rumen

Pfander and Phillipson (38) used the emptied, isolated rumen technique described by Danielli et al. (19) but modified it so that a mixture of volatile fatty acids was continually added to the rumen, approximating the

constant production found under most normal conditions. By adjusting the concentration of the acids in the mixture added, the concentrations in the rumen were held constant. The rate of infusion thus became the index of absorption rate at normal concentrations. The solution introduced into the rumen was Krebs-Ringer bicarbonate solution with sodium salts of acetic, propionic, and butyric acids added to give concentrations of 63, 21, and 15 m-moles per liter respectively. A similar solution containing different proportions of the three acids was added to the rumen. The actual losses from the rumen, in order of magnitude, were acetic > butyric > proprionic. A comparison of the rations added and the rations in the rumen at the beginning of the experiment indicated that the long chain acids disappeared at higher rates relative to their concentration when compared to acetic acid.

Annison, Hill, and Lewis (2) added 50 m-moles of the sodium salts of the volatile fatty acids to the rumen of a similar preparation at one-hour intervals for five hours, then added 100 m-moles one hour later. Fatty acid concentrations were compared 30 minutes after addition of the sodium salts. Doubling the amount added resulted in

slight increases in the rumen concentration of acetic and propionic acids and a pronounced increase in the concentration of butyric acid.

Smyth's laboratory (9, 42) studied the transport of short-chain fatty acids *in vitro* and found that higher concentrations of fatty acids suppressed active transport. They reported an increase in amounts of volatile fatty acids lost from the mucosal side of intestinal epithelium with increasing mucosal concentrations until a maximum was reached. With further increases in concentration, there were decreases in amounts lost from the mucosal fluid. These maxima were observed at lower concentrations as the length of the volatile fatty acid carbon chain increased. The maximum mucosal losses were, in m-molar initial concentrations, acetic, 60; propionic, 40; butyric, 20; valeric, 20; and hexanoic, 10. The serosal gain maxima, in the same order, were observed at 40, 40, 20, 20, and 10 m-molar initial concentrations. After reaching maxima, further increases in mucosal concentration sometimes reduced transport by almost one half. They were unable to observe this suppression of transport *in vivo*.

A possible parallel to suppression of epithelial transport may be noted in the work of Annison et al. (2) where the addition of sodium butyrate caused a large increase in rumen butyrate concentration when compared to the increase when equal amounts of acetate and propionate were added.

Another aspect of concentration effect on volatile fatty acid disappearance from the rumen is the interaction of ionic form of the volatile fatty acid and hydrogen ion concentration. When determining the effect of concentrations under conditions whereby changes in the relative proportion of the ionic forms will be changed, the concentrations and proportions of the form must be specified. These factors and conditions will be discussed in the section on effect of pH.

Effect of Hydrogen Ion Concentration on Volatile Fatty Acid Disappearance from the Rumen

Change in rumen pH value may affect rumen volatile fatty acid absorption three ways: 1) Physical changes in rumen epithelium brought about by the action of the

hydrogen ions, 2) Changes in the proportions of the ionic forms of the volatile fatty acids, and 3) Changes in the animal's metabolism caused by the altered acid-base balance of the blood, such as increased rumen and portal vein blood flow.

At normal rumen pH values, physical changes in rumen epithelium caused by changes in pH value have not been shown to be detrimental to rumen absorption. A condition known as parakeratosis has been shown to be due to feeding rations high in readily fermentable carbohydrates, with a subsequent reduction in rumen pH value. It has not been shown that the changes in the rumen epithelium are due to changes in rumen pH value *per se* or that the changes in rumen epithelium interfere with rumen absorption.

Discussion of the second way hydrogen ion concentration affects absorption of volatile fatty acid must begin with the consideration of the changes in proportion of the two ionic forms, anions and free acid, that exist in the rumen at normal rumen pH values. Changes in concentration of total volatile fatty acid may be slight in relation to the changes in relative concentrations of the

two forms caused by changes in pH value. At pH 7.0 only about 0.5 percent of the total acid is in the unionized form.

Danielli et al.'s (19) original experiments on the effect of pH value on volatile fatty acid absorption led them to propose the hypothesis that the rumen wall is composed of water-filled channels in a fatty membrane. Dobson et al.'s (21) detailed study of the histological organization of the rumen epithelium does not show the existence of water-filled channels. Lavker et al. (31), using an electron microscope, found the stratum corneum and stratum granulosum to be quite similar to the cellular arrangement of the epidermis. Lindhé and Sperber cited by Annison (1a) demonstrated with the electron microscope the presence of intercellular spaces of about 0.1μ. In addition to physical evidence, experimental evidence may be used to show the validity of the water-filled channels:lipid membrane model. Danielli et al. (19) concluded that at pH 7.5 only the fatty acid anion was absorbed, that there was no unionized fatty acid absorbed, and that the fatty acid anion was accompanied by roughly a molecular equivalent amount of sodium. This loss of the fatty acid anion

is via the water-filled channels, the diameter of which are effectively large in proportion to the size of the butyrate molecule. They speculated that these channels are probably in the intercellular cement of the rumen epithelium. After further studies with the rumen contents at pH 5.8, they found a large loss of unionized acid from the rumen in addition to the fatty acid anion loss. The theory advanced was, that though some of the associated acid loss was via the water-filled channels, the greater part of it was via the lipoidal membrane surrounding the channels. As a consequence of the relative importance assigned to these two means of transfer and differences in dissociation constants, at alkaline pH values the volatile fatty acids would be lost in this order: acetate > propionate > butyrate. At acid pH values the order would be reversed: butyrate > propionate > acetate. These differences would come as a result of the differential solubilities of the different acids in aqueous and lipid media as well as the differences in pK_a.

The results of Danielli et al. (19) were challenged by Gray (22, 23) and Gray and Pilgrim (24) who could find no significant losses from the rumen at alkaline pH values.

They attributed the results of Danielli's group to the less sensitive analytical techniques employed. Gray found a 96 percent recovery in the rumen after six hours with his own technique, and 89 and 109 percent recovery with Danielli et al.'s technique.

Ash (6), using acetate, propionate, and butyrate buffers, found changes occurring at pH 3.6 to 4.0 that indicated rapid absorption of free acid. Later, Ash and Dobson (7) investigated this problem from a different cause:effect relationship. Their investigations of effect of absorption upon acidity of the rumen contents indicated that about one half of the acetic acid was absorbed from the rumen in the unionized form and one half as the anion, even though only about 0.5 percent of the free acid is unionized at a neutral pH. Because the concentration of free acid is low, below one m-mole per liter, they surmised that a boundary within the epithelium must be much more permeable to the free acid than to the anion. Because the proportion of acid is quite small at neutrality, Danielli et al. (19) had assumed that they were studying the permeability of the rumen wall to the anion alone. The results reported by Ash and Dobson (above) render this

assumption unjustified. These observations indicated that as larger amounts of free acid became available, that is, as the pH approached the pK_a of a particular acid, the amounts of that volatile fatty acid passively absorbed would increase.

Stevens and Stettler (46) increased the proportion of unionized form tenfold by decreasing pH and compared results from these trials with the results obtained by increasing the total volatile fatty acid concentration threefold. Both changes caused a doubling of acetate disappearance but only a 1.5 fold increase in butyrate disappearance. These results indicate that disappearance is not strictly proportional to amount of free or unionized acid available.

Factors Influencing Appearance of the Volatile Fatty Acids in the Portal Vein

Appearance of material in the portal vein is the second observable event of a complex sequence of events in absorption of material from the rumen. Prior to its appearance in the portal blood flow, the material, or its precursors, must first have disappeared from the rumen

and any factor that affects rumen disappearance will affect appearance of the material in the portal blood flow. This section will deal only with those items that affect the processes that occur subsequent to disappearance from the rumen.

Effect of Volatile Fatty Acid Chain Length on Appearance in the Portal Blood

Both volatile fatty acid chain length and rumen epithelium metabolism exert significant effects on appearance of the volatile fatty acids in portal blood. These effects were first discovered with *in vivo* procedures and later confirmed *in vitro*.

Barcroft, McAnally, and Phillipson (8) studied the relative amounts of volatile fatty acids in the rumen veins, and reported that absorption was inversely related to chain length, a result confirmed by Danielli *et al.* (19) and Masson and Phillipson (33). Masson and Phillipson measured both portal concentrations and disappearance from the rumen and reported that volatile fatty acid concentrations in the rumen vein blood were not proportionate to the amounts of the acids leaving the rumen.

Kiddle, Marshall, and Phillipson (30) reported that rumen vein blood contained proportionately more acetate and less butyrate than the rumen contents, even when the rumen vein concentrations were corrected for arterial amounts of acetate. Pennington (37), using observations on the change in proportions of butyrate and acetate as a basis for further work, has shown rumen epithelium to selectively metabolize butyrate. Bensadoun et al. (10) reported that the ratio of acetate to propionate in the portal blood of fed sheep was greater than the ratio of the two acids in the rumen fluid, this result indicating that propionate is more extensively metabolized than acetate in the rumen epithelium.

Stevens and Stettler (47), using an in vitro system, found less butyrate transported than either acetate or propionate when all were added to the mucosal side in equimolar amounts and reported molar percentage serosal accumulations of 44, 40, and 16 for acetate, propionate, and butyrate, respectively. In an earlier paper, Stevens and Stettler (50) compared mucosal losses and serosal accumulations of acetate and butyrate and found nearly three times as much butyrate as acetate left the mucosal

fluid, but the relative amounts accumulating on the serosal side were almost reversed. Most of these differences were reflected in the amounts of ketone bodies recovered.

Hird and Weidemann (25) found that rumen epithelium *in vitro* releases B-hydroxybutyrate preferentially on the blood or serosal side, regardless of whether it was exposed to the mucosal or serosal side. This unidirectional effect has not been shown in the metabolism of other volatile fatty acids.

Influence of Rumen Concentration on Volatile Fatty Acid Appearance in the Portal Blood

The influence of rumen volatile fatty acid concentration on volatile fatty acid appearance and concentration in the portal blood was first investigated by Masson and Phillipson (33), who found that acetic acid passes into the blood more rapidly at lower concentration than did either propionic or butyric acids. The work of Annison *et al*. (2) indicated the close dependence of the volatile fatty acid concentration in the portal blood on the volatile fatty acid concentration in the rumen.

Stevens and Stettler (46) increased the volatile fatty acid concentration from 30 to 90 m-moles per liter and with acetate both the serosal gain and the mucosal losses made approximately the same increases. With butyrate, the mucosal losses made only about half the relative increase, while the serosal gain was somewhat more than the relative or threefold increase.

Influence of Rumen pH Value on Volatile Fatty Acid Appearance in the Portal Blood

Dobson and Phillipson (20) reported that increased concentration of volatile fatty acids, as well as decreasing rumen pH value with volatile fatty acids present, caused an increase in rumen blood supply. An increased blood supply would effectively increase the concentration gradient between rumen contents and blood. Two later groups of investigators (11, 59) found that both portal blood flow and rumen volatile fatty acid concentration attained maximum levels three to nine hours after feeding.

The differences in mucosal disappearance resulting from changing proportion of ionic form versus increasing total concentration found by Stevens and Stettler (46)

were not paralleled directly in serosal accumulations. Instead they found both increased total acid and increased unionized acid resulted in three to fourfold increases in serosal butyrate accumulation, whereas acetate serosal accumulation did not increase as much with the increased unionized form as it did with increased total concentration. Increased disappearance and serosal accumulation at lower pH values could result from either the increased transepithelial concentration gradient of the unionized form or a greater tissue permeability to this form.

Summary of Literature

Problems that must be considered with *in vivo* studies of rumen absorption include variable pool size, heterogeneity of rumen contents, control or replacement of saliva, rumen gasses, outflow through the omasal orifice, water movement through the rumen wall, and epithelial metabolism.

Techniques for measuring absorption from the rumen use either disappearance from the rumen or appearance in the rumen veins or the portal vein as criteria of

absorption. As both have disadvantages, the technique used will depend upon the purpose of the experiment.

In order to gain more control over some problems of rumen absorption, some experimenters have turned to rumen pouch, isotopic, perfused rumen, and *in vitro* methods.

Absorption from the rumen may be divided into two observable events, disappearance from the rumen and appearance in the rumen or portal vein blood.

Disappearance from the rumen is dependent upon volatile fatty acid chain length and concentration, and hydrogen ion concentration. In general disappearance is inversely proportional to chain length, increases with concentration, and increases with decreased pH value.

These same factors, modified by selective epithelial metabolism, affect appearance in the rumen or portal vein blood.

METHODS AND MATERIALS

In order to study the transfer of material across the rumen epithelium with as simple a system as possible, an in vitro system was developed and used. A forerunner of this device was described by Vidacs, Ward, and Nagy (58) and used to study transfer from rumen fluid to blood plasma.

The device consisted of two Corning[1] 6780-glass ring seal joints, 2 cm in diameter, with synthetic rubber O-rings as seals. Each joint was bent and when two were held together by Corning #32430 clamps, they formed a U-tube with the rumen epithelium clamped across the base as a diaphragm, with an area of 3.14 cm^2. The upper ends of the tubes were stoppered with #3 rubber stoppers, through which passed three polyethylene P.E. 240[2] tubes. One of these carried gas in, extending close to the epithelial diaphragm to insure both saturation of the fluid

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[1]Corning Glass Works, Corning, New York.

[2]Clay-Adams, Inc., New York, New York.

with the gas and mixing of the fluid. A second, which did not reach the surface of the solution, served as a vent for the escaping gas. The third tube, also extending close to the epithelium, terminated above the stopper with a 15-gauge hypodermic needle which served as a connector for sampling.

The assembled U-tubes with their clamps were held upright in a wire basket. The wire basket was then submerged in a water bath at 39°C. The general experimental procedure was as follows: Krebs-Ringer bicarbonate (Appendix I) was brought to 38°C in an insulated container and equilibrated for 15 minutes with a 95 percent oxygen-5 percent carbon dioxide gas mixture at the laboratory. Simultaneously with the departure of one worker for the tissue, another proceeded to prepare the various bathing fluids according to the experimental protocol. For these fluids, Krebs-Ringer bicarbonate was used for both the fluid bathing the lumen (mucosal side) and the muscle (serosal side) sides of the tissue. Additions of materials studied and pH adjustments were made at this time. The fluids were placed in glass and sealed with plastic sheeting to reduce gas exchange pending arrival of the tissue.

Sections of rumen wall, approximately 15 cm square, were removed from the ventral sac of the rumen as soon as it would not interfere with abattoir operation. Samples were selected with papillae from ca .25 to 1.0 cm in length. The rumen wall sections were rinsed once with warm tap water, then placed in an insulated container of oxygenated Krebs-Ringer bicarbonate for transporting back to the laboratory.

When tissue was obtained from the Large Animal Clinic, a non-fasted animal from the Michigan State University dairy herd with a functional rumen was used. A laparotomy was performed to remove a section of the caudo-dorsal blind sac of the rumen. Upon excision of the tissue, it was taken immediately to the laboratory.

When tissue from either source arrived at the laboratory, the transporting fluid was gased with the oxygen-carbon dioxide mixture for three minutes, and then the tissue was rinsed twice in freshly oxygenated Krebs-Ringer bicarbonate. The epithelium was then dissected free of the muscle layers, using as little tension as possible. The epithelium was kept moist by dissecting under oxygenated Krebs-Ringer bicarbonate.

The epithelium was cut into squares large enough to cover the end of the glass joint and clamped in place. The fluids, 40 milliliters each, were added to the appropriate reservoir on either side of the tissue. Fluids on the serosal side were immediately gassed with the oxygen-carbon dioxide mixture. After all U-tubes were positioned and oxygenated, the mucosal fluid was gassed with carbon dioxide. Both the carbon dioxide and the oxygen-carbon dioxide mixtures were first passed through water to saturate them in an attempt to reduce loss of fluid by evaporation.

The time elapsed between stunning the animal in the abattoir and oxygenation of the epithelial tissue was approximately one hour. The time elapsing between excision of the rumen wall by biopsy and oxygenation was about 20 minutes. However, the blood supply had been interfered with to some extent for up to 15 minutes prior to excision.

Zero-time was designated as five minutes after oxygenation was initiated in the last of the U-tubes. Amounts of material in samples taken at this time were subtracted from final amounts to adjust for materials

adhering on membrane surface, or to confirm the presence of tears or cuts in the epithelium, etc. In the usual experimental procedure, the fluids on either side of the epithelium were gassed for the two-hour experimental period, samples being withdrawn at intervals for analysis. Tissues were used for only one two-hour experimental period.

The tissues were incubated in groups of ten aliquots from one animal. All treatments to be compared were represented with the ten aliquots and treatments were replicated if possible.

Hydrogen ion activity was measured as pH units using either a Beckman Model G[3] pH meter or a Coleman Medallion[4] pH meter.

Samples were analyzed for volatile fatty acids by gas-liquid chromatography. A three μl sample acidified to pH 2 to 3 was injected into a 200°C injection port connected to a 2.75 meter length X 2 mm teflon tube packed

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[3]Beckman Instrument, Inc., Fullerton, California.

[4]Coleman Instrument Co., Maywood, Illinois.

with 10 percent FFAP[5] on Chromasorb[6] W, DMCS[7] Acid washed, 80/100 mesh. A flame ionization detector, burning hydrogen and air, ionized and quantified the volatile fatty acids as they left the column in the nitrogen carrier gas. The oven containing the column was operated at 140°C. Two instrument systems were used during the course of the work. The first system consisted of an Aerograph[8] gas chromatograph coupled to a Sargent[9] recorder, using either a one or two mv range plug. An Aerograph steam generator[10] was used with this system. The alternate system was

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[5]FFAP is the Varian Aerograph designation for Carbowax 20M treated with 2-nitroterephthalic acid.

[6]Chromasorb "W" is a white diatomaceous earth material which was flux-calcined with about three percent sodium carbonate and has a surface area of one to 3.5 m^2/g.

[7]DMCS-treated Chromasorb is a Chromasorb support that has been coated with dimethyldichlorosilane to reduce the surface active sites of the diatomaceous earth material.

[8]Hy Fi Model 600, Wilkins Instrument and Research, Inc., now Varian Aerograph, Walnut Creek, California.

[9]Model SRL, E. H. Sargent & Co., Chicago.

[10]Model 675, Wilkins Instrument and Research, Inc., now Varian Aerograph, Walnut Creek, California.

composed of an Aerograph oven,[11] with flame detector connected to a Beckman electrometer.[12] This system used a recorder[13] with one mv full-scale travel. A locally fabricated gas saturator was used to saturate the nitrogen carrier gas with water. The results were quantified by measuring the peak heights and comparing them to a standard curve constructed from standards injected into the gas chromatograph. Peak heights were compared only when all parameters were the same (machine, date, temperature of oven, injector, and room, instrument setting, and operator).

Amounts of volatile fatty acids added, changes in pH, glucose additions, and other experimental variables will be detailed with the individual experiments.

Back transfer was checked in a series of experiments with C^{14}-sodium butyrate. In these experiments 0.2 μCi of 1- C^{14}-sodium butyrate in the normal 40 ml. of

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[11]Model 550, Wilkins Instrument and Research, Inc., now Varian Aerograph, Walnut Creek, California.

[12]Beckman Instrument, Inc., Fullerton, California.

[13]Brown Electronic Model Y153X18(V)-II-III-(118)-(V)[10], Brown Instrument Div., Minneapolis-Honeywell Reg. Co., Philadelphia, Pa.

Krebs-Ringer bicarbonate were placed on the serosal side of the tissue. Unlabeled sodium-butyrate in concentrations of 10, 20, 30, and 40 mM were added to the mucosal side. Tubes were incubated as described earlier. At the end of the two-hour period, the contents were treated as in other experiments except that one milliliter samples from serosal and mucosal fluid were counted by liquid scintillation. The samples were added to 15 ml of Werbin's solution (61), equilibrated at 32°F., and counted three times at 20 minutes each in a 720 System liquid scintillation counter.[14] Then 1046 dpm of standard C^{14}-benzoic acid was added to each vial and the vials were recounted three times at 20 minutes each from which counting efficiency was calculated.

The results were analyzed according to standard methods as outlined by Lewis (32) and Snedecor (43).

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[14]Nuclear-Chicago, Division of Nuclear-Chicago Corporation, Des Plaines, Illinois.

RESULTS AND DISCUSSION

Effects of Changing Mucosal Concentrations on Serosal Accumulations

The first question to be investigated was the influence of mucosal concentration of volatile fatty acids on their serosal accumulation. To study this effect sodium salts of volatile fatty acids were added to the mucosal solution to a final concentration of 20, 40, 60, or 80 mM in the case of acetate and 10, 20, 30, or 40 mM for propionate and butyrate. After a two-hour experimental period, samples were removed from the U-tubes, acidified, and volatile fatty acids quantified. Volatile fatty acids other than the one added to the mucosal fluid were not detected. In conjunction with other trials, three different tissues were incubated without mucosal fatty acid and no volatile fatty acids were detected in the serosal fluid at the end of two hours.

Results for the incubations with acetate, propionate, and butyrate are given in Tables 1, 2, and 3, respectively. The three tissues incubated with acetate

TABLE 1

EFFECT OF MUCOSAL CONCENTRATION ON SEROSAL ACCUMULATION OF ACETATE

Mucosal concentration Sodium Acetate		Serosal accumulation	Confidence Interval ($P < 0.05$)
mM	n	-------μ-moles/2 hr.-------	
20	6	8.4	±1.4
40	7	11.1	±0.4
60	8	15.5	±1.4
80	8	26.5	±4.5

TABLE 2

EFFECT OF MUCOSAL CONCENTRATION ON SEROSAL ACCUMULATION OF PROPIONATE

Mucosal concentration Sodium Propionate		Serosal accumulation	Confidence Interval ($P < 0.05$)
mM	n	-------μ-moles/2 hr.-------	
10	8	4.3	±1.2
20	7	7.2	±0.9
30	6	11.6	±1.9
40	8	19.2	±3.4

TABLE 3

EFFECT OF MUCOSAL CONCENTRATION ON SEROSAL ACCUMULATION OF BUTYRATE

Mucosal concentration Sodium butyrate	Serosal accumulation	
mM	-------μ-moles/2 hr.-------	
	Trial A	Trial B
10	1.28, 1.24	0.63
20	2.30, 3.52	1.16
30	6.60, 6.70	2.80
40	4.50	2.91

responded uniformly so that results from all three tissues could be combined. Similarly, the results from incubation of three different tissues with propionate could also be combined. In contrast to the results with acetate and propionate, the two tissues used with butyrate differed by twofold. Nothing was known of the treatment of the animals prior to slaughter, differences in pre-slaughter conditioning may have contributed to the differences in serosal accumulations. Fasting would be expected to lower the reserves of metabolizable material within the rumen and the rumen epithelium, causing the epithelium to retain more butyrate and release more of it as ketone bodies. Armstrong and his colleagues (4, 5) reported that a

mixture of acids was absorbed more rapidly in sheep previously fasted four days than in fed sheep. Because these workers used disappearance from the rumen fluid as criteria of absorption, they were not able to define the time when volatile fatty acids started appearing in the blood.

The graph (Figure 1) of the serosal accumulations of the volatile fatty acids as a function of their mucosal concentrations has four features to discuss. They are:

1) The differences in magnitude or amounts of the serosal accumulations of the volatile fatty acids.

2) The differences in slopes or sample regression lines of the different volatile fatty acids.

3) The departure of the sample regression lines from linear regression, indicating changing amounts of accumulation with increasing mucosal concentrations.

4) The intersection of the lines representing propionate and acetate accumulations.

The first feature, the differences in magnitude, was examined by testing the differences between the mean

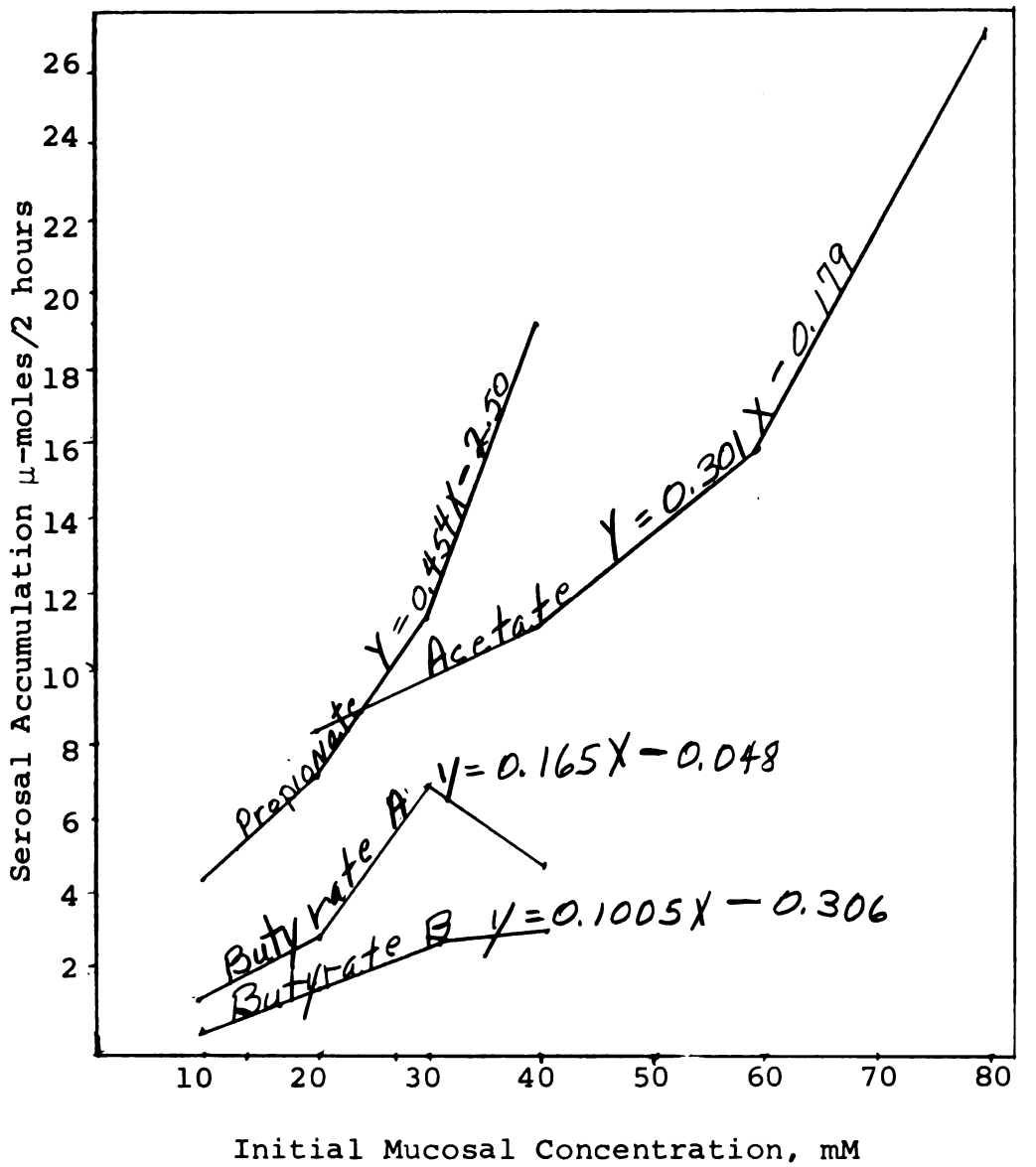

Fig. 1.--Serosal Accumulation of Volatile Fatty Acids as a Function of Mucosal Concentration.

accumulation of the volatile fatty acids at the same mucosal concentrations according to the method given by Lewis (32). The results are shown in Table 4.

TABLE 4

DIFFERENCES IN MEANS OF SEROSAL ACCUMULATIONS OF THE VOLATILE FATTY ACIDS

Acids Compared	Mucosal Concentrations	Level at which means are different
	mM	P <
Acetate vs. Propionate	20	.10
Acetate vs. Propionate	40	.01
Acetate vs. Butyrate A	40	.01
Propionate vs. Butyrate A	10	.10
Propionate vs. Butyrate A	20	.01
Propionate vs. Butyrate A	30	.01

Lower serosal accumulation with increasing carbon chain length at 20 μ-mole/ml mucosal concentrations may indicate differences in relative rates of metabolism of the volatile fatty acids. At lower mucosal concentrations, butyrate accumulates in the serosal fluid at the lowest rate. At 20 μ-moles/ml mucosal concentration, these

comparisons indicate a much greater transfer relative to concentration with the shorter chain lengths in agreement with Barcroft et al. (8).

Kiddle et al. (30) found that the rise in concentration of butyrate in the blood draining the rumen was significantly less than the decline in rumen concentration of butyrate. In recent in vitro work, Stevens and Stettler (46) found that of 16 μ-moles of butyrate lost from the mucosal solution per cm^2 per 2.5-hour experimental period, only about 6 percent reached the serosal solution as butyrate, with about 50 percent being released as ketone bodies, chiefly acetone. Hird and Weidemann (25) also found a large proportion of butyrate metabolized to ketones with in vitro preparations.

A similar case seems to be true for propionate, though to a lesser extent. Stevens and Stettler (46) found that as much as 85 percent of the butyrate lost from the mucosal bath was metabolized, but only 60 to 70 percent of the propionate lost from the mucosal fluid was metabolized. About 88 percent of the butyrate appeared as ketones, but none of the propionate reappeared as ketones. Lower tissue metabolism could account for the

higher relative transfer of propionate than butyrate at the lower mucosal concentrations. The higher relative transfer of acetate at the 20 μ-moles/ml mucosal concentrations would be a reflection of the lower tissue metabolism of this fatty acid (Fig. 1).

The second feature is the different slopes of the lines representing the serosal accumulations of the different acids. When the serosal accumulation values are used in a least-squares formula, the line is described by the equation:

$$y = mx + b.$$

The lines representing the volatile fatty acid accumulations are represented by the following equations:

		r =
Acetate	$y = 0.3013\ X - 0.1788$	+0.88
Propionate	$y = 0.454\ X - 2.495$	+0.93
Butyrate A	$y = 0.1655\ X - 0.0484$	+0.79
Butyrate B	$y = 0.1005\ X - 0.306$	+0.52

The slope, or sample regression coefficient, describes the serosal accumulations as a function of increasing X, or mucosal concentration. As the slopes of these lines are all positive, they indicate that with

increasing mucosal concentration, more of the volatile fatty acids will accumulate in the serosal reservoir. The magnitude of the term indicates the rate of change with increasing mucosal concentration. The value of 0.454 for the propionate slope indicates that propionate accumulations will increase more rapidly with increasing concentrations than either acetate or butyrate. The values for the correlation coefficient, "r" for acetate, propionate, and butyrate A indicate that the regression coefficients for these acids are significantly different from zero. Further analysis indicates a significant ($P < 0.01$) difference between the acetate and propionate regression coefficients. Due in part to the small number of values, the butyrate A regression coefficient could not be distinguished from those of acetate and propionate.

These results seem to confirm the findings of Pfander and Phillipson (38) in that increasing mucosal concentrations increased the transfer of the longer chain acids to serosal solutions more than it did the shorter chain acids, at least in the comparison of acetate and propionate. The present study does not completely parallel Pfander and Phillipson because appearance was

measured, not disappearance. The smaller sample regression coefficient with the increase in chain length can also be attributed to a change in the proportions of material passing through the water-filled pores and the lipid membrane proposed by Danielli (19). The amount passing through the water-filled pores may indeed change only in proportion to the larger amounts available, but the greater lipid solubility of the longer chain acids may allow a larger amount to pass through the lipid membrane resulting in higher serosal accumulations of the longer chain acids. Annison et al. (2) found that differences in portal concentration were greater in the longer chain volatile fatty acids than with acetate with equivalent rumen concentration changes. When the changes in portal concentration are divided by the changes in ruminal concentrations necessary to produce them, there is a progression of 0.70, 0.86, to 1.00 for acetate, propionate, and butyrate, respectively.

The butyrate accumulations were smaller, both in amount and increase with concentration. The smaller amount probably represents greater metabolism by the epithelium. The smaller regression coefficient indicates

less dependence upon concentration, showing that the metabolic pathways are using enough of the available butyrate so that serosal accumulations are almost independent of concentration. If the decrease in serosal butyrate accumulations at 40 m-molar initial mucosal concentrations is real, this would be a parallel to the inhibition of active transport of volatile fatty acid observed by Barry and Smyth (9). Stevens and Stettler (47) found that butyrate disappeared in vitro from the mucosal fluid two to three times as fast as acetate at pH 7.4. However, they found only about 23 percent of the acetate disappearing from the mucosal side appeared as volatile fatty acid in the serosal fluid. They also found somewhat more than three times as much acetate appearing as the volatile fatty acid, indicating greater epithelial metabolism with butyrate.

Despite the general inadvisability of extrapolating regression values beyond the range of values covered by the data, it is interesting to note that the b or intercept term has a negative coefficient. Though a negative concentration is meaningless, it does point out the probability that at low concentrations all the available

volatile fatty acid could be used for epithelial metabolism. The intercepts were tested statistically and were not significantly different from each other or from zero.

The third feature on Figure 1 is the increasing slope with increasing mucosal concentrations. To test the significance of this deviation from linear regression, the sum of squares for group means was partitioned according to the method of Snedecor (43b) into two parts, one attributed to linear regression, the other to deviations from linear regression. When the F-ratios were tested, they indicated more than random sampling departure from linear regression. These calculations are shown in Table 5.

The significant F ratios due to concentration and deviations from linear regression indicate that, in addition to a significant effect of concentration, there is at least one other factor influencing serosal accumulations. As a strict semi-permeable membrane would only be affected by concentration, other explanations are needed. Of the other explanations, three are most likely:

1) Changes in permeability of the aqueous layer to the anion.

TABLE 5

ANALYSIS OF SUMS OF SQUARES FOR GROUP MEANS[1]

Source of Variation	Degrees of Freedom	Mean Square
Acetate Concentration	3	478.0
Linear Regression	1	
Deviations from linear regression	2	69.52
Error	25	9.48
F ratio 69.52/9.48 = 7.33**		
Propionate Concentration	3	343
Linear Regression	1	
Deviations from linear regression	2	185.83
Error	25	6.01
F ratio 185.83/6.01 = 30.92**		

**P < 0.01.

[1]According to Snedecor (43b).

2) Changes in permeability of the lipid layer to the unionized form.

3) Saturation of the volatile fatty acid utilizing pathways in the epithelial cell, resulting in more passage across to the serosal reservoir.

Saturation of the metabolic pathways in the epithelial cell is the more plausable explanation. Propionate is more likely to be metabolized within the epithelium than acetate and also propionate had a greater departure from linear regression in the range of mucosal concentrations studied. Due in part to the smaller number of values, the test for the two butyrate series did not show a significant departure from linear regression.

An attempt to reduce the departure from linear regression by using the logarithms of the accumulation values and testing them by the same method was unsuccessful in reducing the deviations from linear regression.

The fourth feature of Figure 1 is the intersection of the lines representing serosal accumulations of acetate and propionate. The lines intersect possibly as a consequence of greater epithelial metabolism of propionate compared to acetate, causing propionate to have a lower accumulation at 20 mM mucosal concentration and the larger value of the regression coefficient of rate of accumulation. Apparently epithelial metabolic pathways for propionate are saturated at 40 μ-moles/ml the next direct comparison, and the mean for propionate accumulation is

greater ($P < 0.01$) than the acetate accumulation mean. This could also be a consequence of the higher lipid solubility of propionate. This changing of relative amounts transferred at different concentrations may explain some of the difference existing in the literature.

Different values in the literature can be explained by this crossing of serosal accumulation lines. For example, Masson and Phillipson (33) found that acetate passed into the blood stream in larger quantities than did the other acids at equally low concentrations. Annison et al. (2) found more rapid increases in portal concentrations with increased ruminal butyrate concentrations. Stevens and Stettler (46) found that at higher mucosal concentrations of butyrate an increase in mucosal concentration resulted in a larger than equivalent increase in serosal accumulation, while under the same conditions, acetate serosal accumulations did not respond to the same extent. All these examples may be taken as indicative of the change in mucosal accumulation brought about by a smaller proportion of longer chain acids being metabolized in the tissues at higher mucosal concentrations and the increased lipid solubility of the longer chain acids.

Comparison of Slaughter- Versus Biopsy-Obtained Tissue

In order to test the viability of the tissues used a comparison of responses of tissue obtained by rumen biopsy with those from slaughter material was made. Rumen biopsies were done on animals leaving the Michigan State University dairy research herd. Experimental conditions were similar for biopsy and slaughter material. Two-hour accumulations with the experimental parameters are shown in Table 6.

The differences between the means of the two tissue sources were calculated and tested according to Lewis (32) and the results shown in Table 6. The significant or highly significant differences between the biopsy and slaughter material, with the slaughter material having a higher accumulation in all cases at pH 7.4 indicates the possibility that the epithelium from the biopsy material is metabolizing more of the available volatile fatty acid. No explanation is offered for the results with acetate at 4.0. Stevens (45) compared slaughter material with biopsy material and found the electrical properties favored the biopsy material. As much of the experimental procedures

TABLE 6

BIOPSY VERSUS SLAUGHTER MATERIAL

Trial	pH	Mucosal Parameter		Serosal Accumulation		Difference Slaughter-Biopsy
		VFA	VFA concentration	Biopsy	Slaughter	
			mM	----------μ-moles/2 hour----------		
1	7.5	Acetate	80	6.74 (10)	13.40 (10)	6.66**
2	4.0	Acetate	80	65.30 (10)	34.60 (5)	-30.70
3	7.5	Propionate	20	6.11 (10)	7.20 (7)	1.09*
4	7.5	Propionate	40	13.10 (10)	19.20 (8)	6.10**

*Sig. difference ($P < 0.05$).

**Sig. difference ($P < 0.01$).

used in his experiments required the improved electrical properties, only biopsy material was used in further experiments. There was no comparison of slaughter material and biopsy material for other properties. The biopsies for his work were taken through a rumen fistula, removing only a small area each time which permitted using rumen tissue from one animal for a series of experiments. Repeated operations were not utilized in this experiment.

Effect of Adding Glucose to Serosal Fluid on Serosal Accumulation of Acetate and Propionate

This series of experiments investigated the effects of adding 29.6 mM glucose to the serosal fluid when acetate and propionate were added to the mucosal fluid at 20, 40, 60, and 80 mM and 10, 20, 30, and 40 mM final concentration for acetate and propionate, respectively.

The aim of this series of experiments was to determine if:

1) Glucose would supply energy to enhance volatile fatty acid transfer.

2) Glucose would spare or reduce the amounts of fatty acids metabolized, allowing more serosal accumulation.

3) Increasing the osmolarity of the serosal fluid would affect serosal accumulation.

Accumulations reported in Table 7 show that glucose tended to inhibit serosal accumulation of volatile fatty acids, although differences between means were not significant. Whether transfer of volatile fatty acids is carrier mediated or not, the addition of glucose to the serosal fluid did not enhance transfer. Thus increased blood glucose would not enhance fatty acid transfer. This may be a protective device, whereby fatty acid absorption is not dependent upon glucose, a source of energy in somewhat short supply.

In answer to the second question, glucose did not spare volatile fatty acid metabolism, as evidenced by no significant differences in the means. The lack of significant differences also negates the third possibility (effects due to osmolarity) especially as no differences were observed in fluid volumes.

TABLE 7

EFFECT OF ADDING GLUCOSE TO SEROSAL FLUID ON SEROSAL ACCUMULATION

	Mucosal Concentration	Serosal Accumulation		Difference Glucose-Control
		Glucose	Control	
	mM	μ-moles/2 hrs.		
Sodium Acetate:	20	6.47	10.40	-3.93
	40	11.11	11.51	-0.40
	60	13.39	17.85	-4.46
	80	26.98	25.17	+1.81
Sodium Propionate:	10	5.06	3.56	+1.50
	20	6.70	11.01	-4.31*
	30	10.25	13.12	-2.78
	40	13.35	21.75	-8.40**

*($P < 0.05$).
**($P < 0.01$).

Effect of Serosal Butyrate

In order to investigate the effect of butyrate on the accumulation of the other volatile fatty acids, 0.8 mM sodium butyrate was added to the serosal side. The comparisons were made with various experimental parameters on the mucosal side, including change in pH, concentrations

of volatile fatty acids, different volatile fatty acids, and combinations of acids.

The results of these comparisons are presented in Table 8. Serosal butyrate had no effect on acetate or propionate accumulation at pH 7.4. Serosal butyrate inhibited both acetate and propionate accumulation at pH 4. In the intact animal there is normally little butyrate circulating beyond the liver. The liver removes almost all of the butyrate appearing in the portal vein. Hird and Weidemann (25) found that sheets of rumen epithelium, in vitro, synthesized greater amounts of ketone bodies when butyrate was presented to the muscle or serosal side than when presented to the mucosal side of their preparation. They suggested that either the muscle side is more permeable to butyrate or that synthesis of ketone bodies is spatially closer to that surface. If the acceptance of acetate into the mucosal surface of the cell was a carrier mediated process or a process dependent upon the metabolism of the cell, the presence of butyrate at the more permeable serosal surface might cause the cell not to accept acetate as rapidly and it would not be available to the serosal side.

TABLE 8

EFFECT OF SEROSAL BUTYRATE ON 2-HOUR SEROSAL ACCUMULATION OF ACETATE AND PROPIONATE

Volatile Fatty Acid	n	Mucosal Concentration mM	pH	With Butyrate Accumulation	Control Accumulation	Difference Butyrate-Control
				-------μ-moles/2 hours-------		
HAc	4	80	7.4	13.5	14.0	- .5
HAc	4	80	4.0	34.6	76.4	-41.8**
HAc	4	80	4.0	33.6	54.0	-20.4
HPr	2	40	7.4	15.4	12.4	+ 3.0
HPr	4	80	4.0	26.3	51.6	-25.3**
HAc/HPr	2	80				
HAc			4.0	32.4	44.6	-12.2
HPr			4.0	28.8	24.6	+ 4.2

**($P < 0.01$).

The effect of serosal butyrate upon the transfer of propionate at pH 7.4 is easier to explain, though the difference only approaches significance. Here the marginal amount of propionate available to the cell is spared by the serosal butyrate.

Effect of Sodium Cyanide on Serosal Accumulation of Acetate

In an attempt to determine if the processes by which the volatile fatty acids were transferred were active or passive, 10^{-3} molar sodium cyanide was added to the serosal side of the membrane in a group of five tubes which were compared with a group of five tubes without sodium cyanide. The fluid on the mucosal side contained 80 mM sodium acetate in Krebs-Ringer Bicarbonate, pH 7.4, and the tubes were incubated for two hours.

The serosal acetate accumulation averaged 25.7 μ-moles/2 hr. NaCN, 24.6 μ-moles/2 hr. without NaCN, a difference of 1.1 μ-mole, which is not significant. There are three possibilities which may be used to explain the results:

1) If an energy dependent process for acetate transport operates in the cell, it is not oxygen dependent.

2) Enough energy is stored as chemical intermediates to support transport in the absence of oxygen.

3) Serosal accumulation is the result of passive transport through the rumen epithelium.

The first two possibilities are not probable. Glycolysis, the normal metabolic pathway when oxygen is not available, would not have any normal substrate in the chemically defined media used. The possibility of a sufficient supply of energy to support transfer as chemical intermediates is unlikely, if not impossible. Because of the wide range of enzymes inhibited by cyanide, specifically those in the cytochrome chain, the rumen epithelium is unlikely to be so different as to have a system unaffected by cyanide. Hird and Weidemann (30) found that oxygen stimulated conversion of butyrate to B-hydroxybutyrate in rumen epithelium. If an active system for mucosal to serosal transport of acetate exists in the rumen epithelium, its effects were small.

In view of the small increase in serosal accumulation with cyanide treatment, cyanide might have blocked the mechanism proposed by Stevens and Stettler (48) for active acetate transport from the blood to rumen. Less back transfer could account for the larger net serosal accumulation.

Effect of Changing pH on Acetate and Propionate Accumulation in Serosal Fluid

To investigate the effect of changing the proportions of ionized and unionized acid in the mucosal fluid, trials were conducted at different pH values. The pH of the mucosal solution was adjusted to a predetermined level by adding sulfuric or hydrochloric acids. These acids were added to the buffer with the volatile fatty acids, before exposing the fluid to the membrane. The results are shown in Table 9.

Changing the pH from 7.4 to 6.0 with sulfuric acid did not cause a significant change in serosal acetate accumulation. Further adjustment of the pH from 6.0 to 4.0 with sulfuric acid caused a significant ($P < 0.01$) increase in serosal acetate accumulation, despite the fact that there was no significant difference in accumulation between the control tissues.

The use of hydrochloric acid to alter the pH also resulted in a significant ($P < 0.01$) increase in serosal accumulation relative to the control. Comparing sulfuric and hydrochloric acids as adjusting agents, there was a larger increase ($P < 0.01$) in serosal accumulation with

TABLE 9

EFFECT OF CHANGING MUCOSAL HYDROGEN ION CONCENTRATION ON SEROSAL ACETATE AND PROPIONATE ACCUMULATION

Adjusting Acid	pH	Serosal Accumulation[1]		Difference Adjusted-Control
		Adjusted	Control[2]	
		----------μ-moles/2 hr.----------		
With 80 mM sodium acetate on mucosal side:				
H_2SO_4	6.0	12.95	14.18	- 1.23
H_2SO_4	4.0	34.6	13.77	20.83**[3]
HCl	4.0	65.4	6.57	58.83**[3]
With 80 mM sodium propionate on mucosal side:				
H_2SO_4	4.0	71.2	13.79	51.41**

[1] n = 5

[2] Krebs-Ringer Bicarbonate at pH 7.4 on mucosal side.

[3] Significant ($P < 0.01$) difference between increases caused by H_2SO_4 and HCl.

**Significantly different ($P < 0.01$).

the hydrochloric acid, despite the significantly ($P < 0.01$) smaller accumulation in control tissues.

The difference in serosal accumulation in the comparison between hydrochloric acid and sulfuric acid as the pH adjusting acid is evidently due to differing

actions of the chloride and sulfate ions on the epithelium. The mechanism of this action is not presently known.

Propionate incubated tissues responded to pH change in much the same way. When tissues exposed to 80 mM sodium propionate adjusted to pH 4.0 with sulfuric acid were compared with tissues under the same conditions at pH 7.4, there was a significantly ($P < 0.01$) larger serosal accumulation at the lower pH value.

Changing pH has at least three ways of affecting serosal accumulation or transfer by rumen epithelium. The first of these is the direct effect of the hydrogen ions on the rumen epithelium. The second is the change in molecular or ionic species of the weak electrolytes in the rumen. The third is an inhibition of epithelial metabolism, with a larger amount passing into the serosal fluid as a result. Between pH 7.4 and 6.0, any effect of changing hydrogen ion concentration on the epithelium itself must be slight. However, pH 4.0 is beyond the range of normal pH values for most tissues.

The change in proportion of molecules existing as the two species of volatile fatty acid brought about

by changing the pH from 7.4 to either pH 6.0 or pH 4.0 is extensive. Free acid increases 25 times from pH 7.4 to pH 6.0 and 100 times from pH 6.0 to 4.0. The changes in serosal accumulation were not of this magnitude.

The question is not so much whether serosal accumulation is 25 to 100 times as much but whether it increases as much as regression equations on Figure 1 would predict. If we may assume that the regression equation for total acid is applicable to free acid, then the increase in free acid caused by reducing the pH is 100 times. The sample regression coefficient, 0.301, times the 100 increase in free acid would be 30, which is less than the 58.8 difference for hydrochloric acid adjusted tissue, but more than the difference for the sulfuric acid adjusted tissue. However, the observed differences were within the precision of the prediction equation.

Ash (6) used acetate, propionate, and butyrate buffers and found changes occurring at pH 3.6 to 4.0 that indicated rapid absorption of the free acid. Ash and Dobson (7) found about one half of the acetic acid absorbed from the rumen as the unionized form and one half as the anion, even though only about 0.5 percent of the

total acid is unionized at a neutral pH. Because the concentration of unionized acid was low, less than a m-mole per liter, they surmised that a boundary within the epithelium must be much more permeable to the free acid than to the anion. These results render unjustified Danielli et al.'s (19) assumption that because the proportion of free acid is reduced to small proportion at neutral pH, only the anion leaves the rumen. Ash and Dobson (7) indicated that as the pH of the rumen fluid approached the pK_a of a particular acid, the amounts of that volatile fatty acid passively transferred would increase.

Stevens and Stettler (46) increased the concentration of the unionized forms of the fatty acids acetate and butyrate tenfold by lowering the pH from 7.4 to 6.4 and found that this change affected the two acids differently. Acetate transport increased 1.5 times under these conditions, while butyrate transport increased three- to fourfold. When these results are compared with the results of changing the amount of sodium salt of the particular fatty acid added to mucosal solution, acetate accumulation increased in proportion to the rise in total

mucosal acid, but when the proportion of unionized form was increased acetate transfer did not increase in proportion to the increase in unionized form.

A sidelight is the close comparison between the amounts of acetate and propionate accumulating (±13.8 μ-moles/2 hr.) under the control conditions of 80 mM at pH 7.4 (Table 9). This was considerably less propionate accumulation than would be predicted by extrapolation from Fig. 1 which may indicate that propionate, as well as butyrate, inhibits serosal accumulation when added to the mucosal fluid at higher concentrations. However, acetate accumulation in Table 9 was slightly less than predicted from Fig. 1.

The differences in serosal accumulations for control tissues, both in this series of experiments and in others where control tissues were necessary, have at least two possible explanations. The first and more probable is the differences in papilla length and epithelial thickness. The longer the papilla or the thicker the epithelia, the longer distance and the more barriers volatile fatty acids must traverse to appear as serosal accumulations. The second reason, somewhat related, is

differences in tissue utilization of volatile fatty acid. Here again, more tissue or greater depth of tissue would metabolize more volatile fatty acid.

Study of Serosal to Mucosal Transfer Using C^{14}-Sodium Butyrate

To study the amount of back transfer, tubes with 0.2 μCi as 13 μ-moles of sodium butyrate-1-^{14}C in the serosal fluid were compared to tubes without serosal sodium butyrate. The mucosal amounts, as shown in Table 10, were 10, 20, 30, or 40 mM unlabeled sodium butyrate. At the end of the two-hour incubation period, samples were taken for liquid scintillation counting as well as for chromatographic analysis.

Addition of 0.325 mM sodium butyrate to the serosal side had no effect on the serosal accumulation of butyrate. This is in contrast to the effect of adding 0.8 mM sodium butyrate on the accumulation of acetate and propionate (Table 8).

While the back diffusion as measured by the radioactivity was small, always less than one percent, it was larger than accumulation of butyrate in the

TABLE 10

SEROSAL TO MUCOSAL BUTYRATE TRANSFER[1]

Sample	Mucosal Butyrate Concentration	Serosal Accumulation[2]	Net CPM/ml[3,4]		Back Diffusion[5]	Retained by Tissue[6]
			Mucosal	Serosal		
	mM	μ-moles/2 hr.			(%)	(%)
1	10	0.62				
2	20	1.16				
3	30	5.55				
4	30	2.80				
5	40	2.91				
6	10	0.67	22.5	6552	0.32	18.5
7	20	1.36	25.2	6007	0.37	24.0
8	30	2.81	52.5	6146	0.80	23.5
9	40	2.99	27.1	6251	0.40	12.2
10	40	2.99	46.2	6314	0.71	18.0

[1] pH = 7.4.

[2] Corrected for 13 μ-moles added as labeled butyrate and carrier.

[3] Average of 3, twenty-minute counting periods.

[4] Background = 52.34 CPM.

[5] Corrected for efficiency.

[6] Calculated by difference.

serosal fluid measured as percent of mucosal butyrate despite the much greater concentration gradient mucosal to serosal. This agrees with the preferential absorption from the muscle side of the tissue found by Hird and Weidemann (25).

Radioactive carbon not accounted for in either fluid was thought to be either absorbed into the tissues or volatilized into the air. This amount ranged from 12 to 24 percent and may be indicative of a proportionally greater tissue uptake from the serosal side. The effect of this back diffusion would be to minimize the amounts of butyrate reaching the liver.

Time Sequences for Serosal Accumulations

An examination of the serosal accumulations at intermediate times for acetate (Table 11) and for propionate (Table 12) and graphs of serosal accumulations as functions of time (Figures 2 and 3) shows two items pertinent to this study.

The first is that there is a time lag before the volatile fatty acids appear in the serosal fluid in measurable quantities. This is probably due to two factors:

TABLE 11

TIME SEQUENCE FOR ACETATE ACCUMULATION

pH	Source	Parameters		Serosal Accumulation at Time			
		Mucosal Concentration	Acid	1/2 hr.	1 hr.	1-1/2 hr.	2 hr.
		mM		----------μ-moles----------			
7.4	Slaughter	80			4.6	12.0	26.8
7.4	Slaughter	60				6.0	15.5
6.0	Slaughter	80	H_2SO_4			7.1	13.0
7.4	Slaughter	80				6.5	14.4
4.0	Slaughter	80	H_2SO_4	4.8	11.0	22.8	34.6
7.4	Slaughter	80				5.7	13.8
4.0	Slaughter	80	HCl	9.2	24.9	43.9	65.4
7.4	Slaughter	80					6.6
7.4	Biopsy	80				4.1	6.7
7.4	Slaughter	80				5.7	13.4
4.0	Biopsy	80	H_2SO_4	7.9	17.9	33.2	65.3

TABLE 12

TIME SEQUENCE FOR PROPIONATE ACCUMULATION

pH	Source	Parameters		Serosal Accumulation at Time			
		Mucosal Concentration	Acid	1/2 hr.	1 hr.	1-1/2 hr.	2 hr.
		mM		----------μ-moles----------			
7.4	Slaughter	40			3.5	12.0	19.2
7.4	Slaughter	30				3.9	11.6
7.4	Biopsy	40				5.7	13.1
4.0	Slaughter	80	H_2SO_4	7.1	14.3	35.2	71.2
7.4	Slaughter	80				6.0	13.8

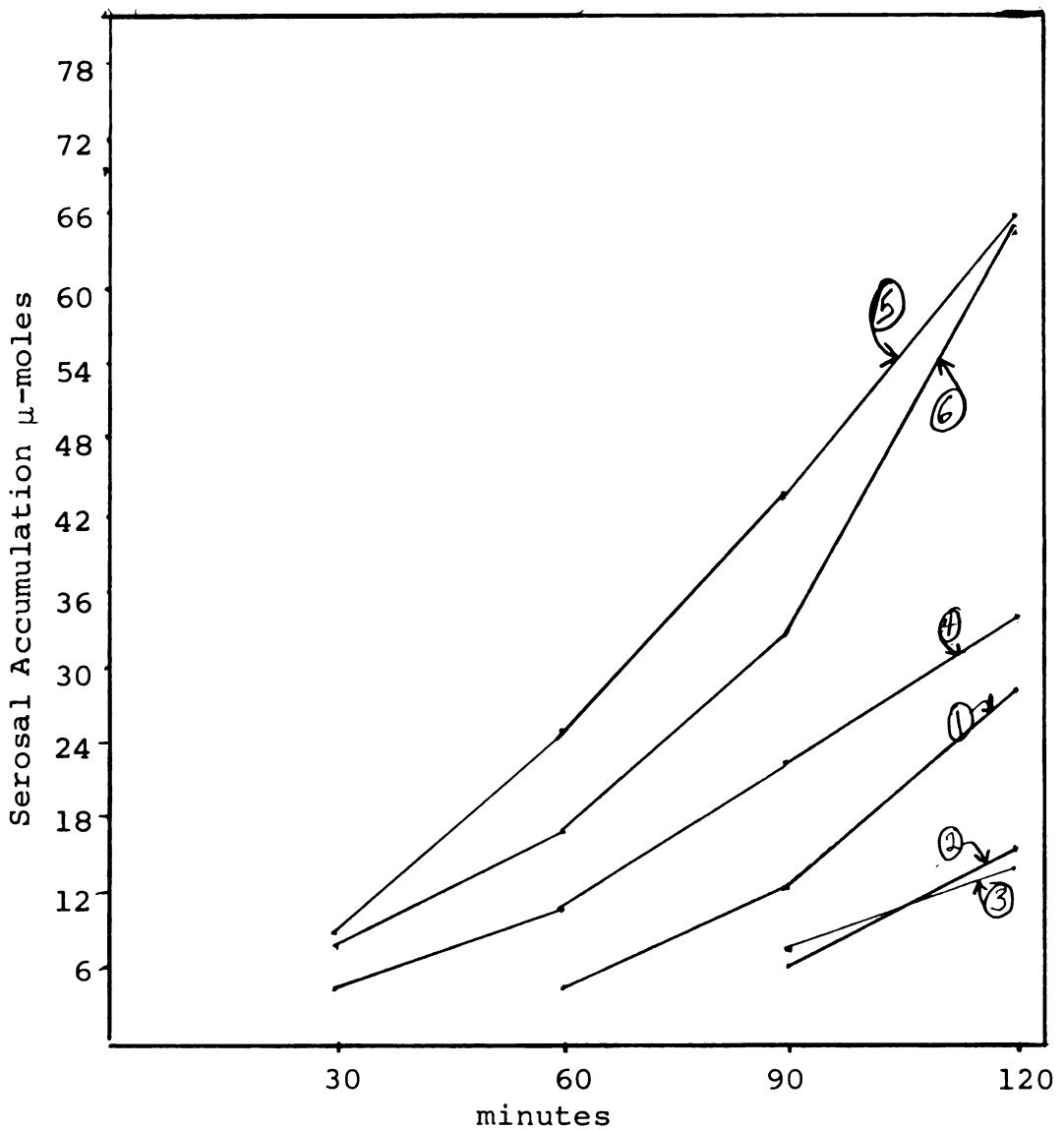

Fig. 2.--Time Sequence of Acetate Serosal Accumulations.

	Source	Mucosal Conc. mM	pH	Acid
1.	Slau	80	7.4	
2.	Slau	60	7.4	
3.	Slau	80	6.0	H_2SO_4
4.	Slau	80	4.0	H_2SO_4
5.	Slau	80	4.0	HCl
6.	Biopsy	80	4.0	H_2SO_4

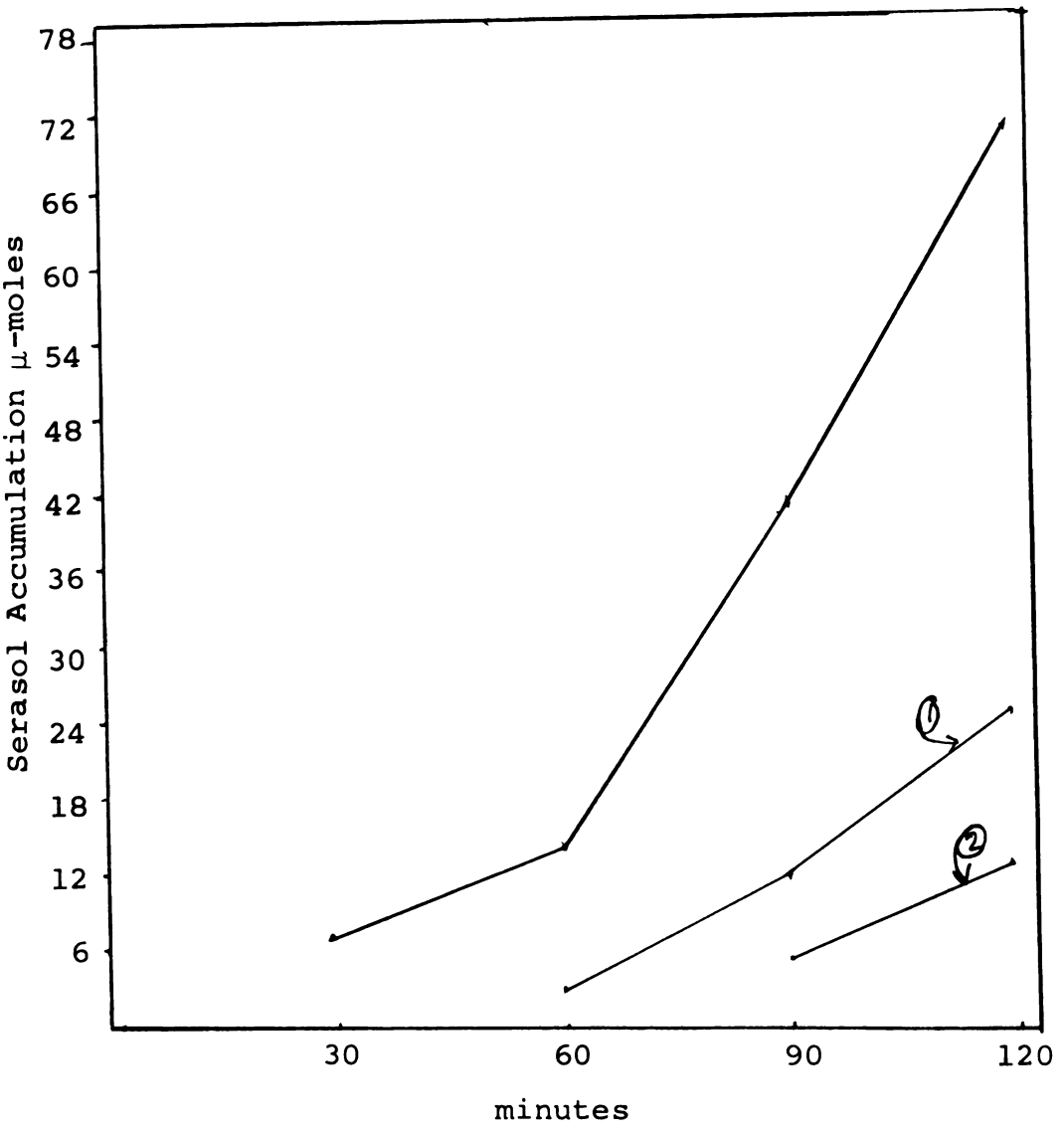

Fig. 3.--Time Sequence of Propionate Serosal Accumulation.

	Source	Mucosal Conc. mM	pH	Acid
1.	Slau	40	7.4	
2.	Biopsy	40	7.4	
3.	Slau	80	4.0	H_2SO_4

1) The fact that the fatty acid ion or molecule, as the case may be, must traverse two membrane surfaces and the distance between them.

2) Any metabolic uses for the fatty acid going through the cells themselves are first satisfied before the excess is available to pass through the serosal membrane.

Lowering the pH of the mucosal fluid increased the total amount transferred and also caused transfer to occur with less time lag. This earlier accumulation with lower pH could reflect larger amounts of unionized fatty acid available to the cell or an inhibition of the cell metabolic processes or both.

The serosal accumulations are not direct measures of transport rate in the usual enzyme kinetic sense of showing maximum reaction rates.

The second feature is the failure to reach equilibrium or a steady state. Normally a graph of reaction products as a function of time consists of three phases: 1) lag, 2) rate, and 3) steady state. In measuring and comparing two hour accumulations we are measuring at least

one and probably at least two of these phases. In the absence of longer than two-hour experimental periods, there is no evidence that the steady state phase has been reached. Further work is indicated to locate these curves on the usual reaction products curve.

CONCLUSIONS

Despite the apparent failure to measure the constant maximum rate of fatty acid transfer due to the time lag, this lag was reduced at higher mucosal concentrations and lower pH values but was independent of fatty acid chain length. Comparisons among fatty acids at two hours are a measure of their relative transfer rates. Within these limitations, the following conclusions are valid.

Passage of the volatile fatty acids through the rumen epithelium was influenced by chain length. The shorter chain length acids accumulated in the serosal fluid at two hours in larger amounts at lower mucosal concentrations, except for acetate vs. propionate at 20 mM. At higher mucosal concentrations propionate accumulated in larger amounts than did acetate. Butyrate accumulation was not appreciably affected by mucosal concentration in the ranges used. These results are consistent with the premise that the volatile fatty acids are transported by a non-active process modified by the metabolism of the

epithelial cells. The metabolic processes become saturated, allowing larger amounts to accumulate. With higher mucosal concentrations more fatty acids accumulate in the serosal fluid possibly because the epithelial metabolic pathways become saturated.

In a comparison of tissue obtained by rumen biopsy and tissue obtained from the abattoir, the slaughter tissue allowed significantly larger serosal accumulation, a result consistent with larger amounts used in metabolic pathways in the tissue obtained by biopsy.

Serosal glucose had no effect on acetate accumulation at two hours and significantly decreased propionate accumulation. Glucose would not be a normal source of energy for the cell and it would not spare volatile fatty acid metabolism by the epithelial cells, or add to its transfer.

Sodium butyrate added to the serosal fluid significantly reduced both acetate and propionate accumulation for the one mucosal concentration and did not increase accumulation with either acetate or propionate.

Sodium cyanide in the serosal fluid did not cause a significant difference in acetate accumulation. The non-significant increase may have been due to either a

reduction in acetate metabolism or a reduction in a serosal to mucosal active transport.

Adjusting the pH to 4.0 significantly increased two-hour serosal accumulation of both acetate and propionate. The increase was larger when hydrochloric acid was used as the adjusting agent than when sulfuric acid was used. The increase in serosal accumulation with the change in pH is probably due to the change in proportion of anion and unionized forms but this does not explain the change in accumulation caused by the change in adjusting agent.

Serosal to mucosal transfer measured with C^{14}-butyrate moved a larger percentage of carbon than did mucosal to serosal transfer.

Back diffusion did not affect other results because of the small amounts available in the serosal fluid.

APPENDIX I

KREBS-RINGER BICARBONATE (56) SOLUTIONS:

			Parts
1.	0.90% NaCl	(0.154M)	100
2.	1.15% KCl	(0.154M)	4
3.	1.22% $CaCl_2$	(0.11M)	3
4.	2.11% KH_2PO_4	(0.154M)	1
5.	3.82% $MgSO_4 \cdot 7H_2O$	(0.154M)	1
6.	1.30% $NaHCO_3$	(0.154M)	21

The solutions were combined in the above proportions, gassed for 10 minutes with 95% O_2-5% CO_2 and kept cold and stoppered until used. Fresh solutions were prepared for each day's experiments.

APPENDIX II

EFFICIENCY DATA FOR COUNTING C^{14} SODIUM BUTYRATE

		CPM/ml.			Efficiency Percentage
		Sample + Background	Sample + Standard	From Standard*	
Solution	1**	51.53	679.83	628.30	60.06
	2**	53.15	635.92	582.77	55.70
Mucosal	6	74.86	687.56	612.80	58.58
	7	77.60	666.28	588.68	56.28
	8	105.06	683.01	577.95	55.25
	9	79.48	658.76	579.28	55.38
	10	98.58	681.60	583.02	55.74
Serosal	6	6604.3	7308.2	703.9	67.29
	7	6059.3	6769.7	710.4	67.92
	8	6198.5	6906.3	707.8	67.67
	9	6303.5	6943.9	640.4	61.22
	10	6368.0	7043.2	677.2	64.74

*1046 DPM added in standard.

**Solution 1 and Solution 2 were Krebs-Ringer Bicarbonate plus scintillation mixture and served as background.

VITA

John Walter Bell was born in Cave Springs, Arkansas, October 9, 1936. He received his elementary and secondary schooling at Pea Ridge, Arkansas, graduating in 1954. He received an Associate in Science in Agriculture from Arkansas Polytechnic College in 1956, and a Bachelor of Science in Agriculture in Dairy Science from the University of Arkansas in 1958. He entered the U.S. Army in 1959 as an officer and served two years. He re-entered the University of Arkansas and received the M.S. degree in Animal Nutrition in 1963 doing his research under the guidance of Dr. O. T. Stallcup. He was a graduate research assistant in the Department of Dairy, Michigan State University, from 1962 to 1967. He is presently Assistant Professor of Agriculture at Western Illinois University, Macomb, and will receive the Ph.D. degree in 1970. He is a member of National Association of College Teachers of Agriculture, American Dairy Science Association, American Society of Animal Science, Alpha Zeta, Gamma Sigma Delta, ODK, and an associate member of Sigma Xi.

REFERENCES

1a. Annison, E. F. in Digestive Physiology in the Ruminant. Page 185. Edited by R. W. Dougherty, et al. Butterworths, Washington. 1965.

1b. Ibid., page 189.

2. Annison, E. F., K. J. Hill, and D. Lewis. Studies on the Portal Blood of Sheep. 2. Absorption of Volatile Fatty Acids from the Rumen of the Sheep. Biochem. J. 66:592, 1957.

3a. Annison, E. F., and D. Lewis. Metabolism in the Rumen. Page 59. Methuen, London. 1959.

3b. Ibid., page 137.

4. Armstrong, D. G., and K. L. Blaxter. The Heat Increment of Steam Volatile Fatty Acids in Fasting Sheep. Brit. J. Nutr. 11:247, 1957.

5. Armstrong, D. G., K. L. Blaxter, and N. McGraham. The Heat Increment of Mixtures of Steam Volatile Fatty Acids in Fasting Sheep. Brit. J. Nutr. 11:392, 1957.

6. Ash, R. W. Inhibition of Reticulo-Rumen Contractions by Acid. J. Physiol. 133:75p, 1956.

7. Ash, R. W., and A. Dobson. The Effect of Absorption on the Acidity of Rumen Contents. J. Physiol. 169:39, 1963.

8. Barcroft, J., R. A. McAnally, and A. T. Phillipson. Absorption of Volatile Fatty Acids from the Alimentary Tract of the Sheep and Other Animals. J. Exp. Biol. 20:120, 1944.

9. Barry, R. J. C., and D. H. Smyth. Transfer of Short-Chain Fatty Acids by the Intestine. J. Physiol. 152:48, 1960.

10. Bensadoun, A., O. L. Paladines, and J. T. Reid. Effect of Level of Intake and Physical Form of the Diet on Plasma, Glucose Concentration, and Volatile Fatty Acid Absorption in Ruminants. J. Dairy Sci. 45:1203, 1962.

11. Bensadoun, A., and J. T. Reid. Estimation of Portal Blood Flow in Ruminants: Effect of Feeding, Fasting, and Anesthesia. J. Dairy Sci. 45:540, 1962.

12. Bloom, B., I. L. Chalkoff, and W. O. Reinhardt. Intestinal Lymph as a Pathway for Transport of Absorbed Fatty Acids of Different Chain Lengths. Am. J. Physiol. 166:451, 1951.

13. Brown, R. E., C. L. Davis, J. R. Staubus, and W. O. Nelson. Production and Absorption of Volatile Fatty Acids in the Perfused Rumen. J. Dairy Sci. 43:1788, 1960.

14. Coe, E. L. Prediction of the Relative Passive-Transfer Rates of Short-Chain Fatty Acids Across Lipid Membranes. Nature 211:80, 1966.

15. Coe, E. L, and M. H. Coe. A Hypothesis Relating Oil:Water Partition Coefficients and Vapor Pressures of Nonelectrolytes to their Penetration Rates through Biological Membranes. J. Theoretical Biology 8:327, 1965.

16. Conver, H. G., D. McGilliard, and C. F. Huffman. Bovine Re-entrant Duodenal Fistula Studies. I. Establishment of Re-entrant Duodenal Fistula. J. Animal Sci. 16:692, 1957.

17. Cook, R. M. Use of C^{14} to Study Utilization of Substrates in Ruminants. J. Dairy Sci. 49:1018, 1966.

18. Cunningham, H. M. Inflatable Plug for Reticulo-Omasal Orifice and Equipment for Saliva Collection from Fistulated Steers. Can. J. Animal Science 38:84, 1958.

19. Danielli, J. F., M. W. S. Hitchcock, R. A. Marshall, and A. T. Phillipson. The Mechanism of Absorption from the Rumen as Exemplified by the Behaviour of Acetic, Propionic, and Butyric Acids. J. Expl. Biol. 22:75, 1945.

20. Dobson, A., and A. T. Phillipson. The Influence of the Contents of the Rumen and of Adrenaline Upon Its Blood Supply. J. Physiol. 133:76p, 1956.

21. Dobson, M. J., W. B. Brown, A. Dobson, and A. T. Phillipson. A Histological Study of the Organization of the Rumen Epithelium of Sheep. Quart. J. Expl. Physiol. 41:247, 1956.

22. Gray, F. V. The Absorption of Volatile Fatty Acids from the Rumen. J. Expl. Biol. 24:1, 1947.

23. Gray, F. V. The Absorption of Volatile Fatty Acids from the Rumen. II. The Influence of pH on Absorption. J. Expl. Biol. 25:135, 1948.

24. Gray, F. V., and A. F. Pilgrim. Fermentation in the Rumen of the Sheep. II. The Production and Absorption of Volatile Fatty Acids During the Fermentation of Wheaten Hay and Lucerne Hay in the Rumen. J. Expl. Biol. 28:83, 1951.

25. Hird, F. J. R., and M. J. Weidemann. Transport and Metabolism of Butyrate by Isolated Rumen Epithelium. Biochem. J. 92:585, 1964.

26. Hydén, S. Observation on the Absorption of Inorganic Ions from the Reticulo-Rumen of the Sheep. Kungl. LantbrHögsk. Annlr. 27:273, 1961.

27. Johnson, R. B. The Effects of the Administration of Acetic, Propionic, and N-Butyric Acids Upon the Blood Glucose and Ketone-Body Levels of Goats. Cornell Vet. 45:273, 1955.

28. Johnson, R. R. Techniques and Procedures for In Vitro and In Vivo Rumen Studies. J. Animal Sci. 25:855, 1966.

29. Kameaka, K. and H. Morimoto. Extent of Digestion in the Rumen-Reticulum-omasum of Goats. J. Dairy Sci. 42:1187, 1959.

30. Kiddle, P., R. A. Marshall, and A. T. Phillipson. A Comparison of the Mixture of Acetic, Propionic, and Butyric Acids in the Rumen and in the Blood Leaving the Rumen. J. Physiol. 113:207, 1951.

31. Lavker, R., J. F. Dickey, and W. Chalupa. Fine Structural Observations on the Stratum Germinativum from Bovine Fore-Stomach. Fine Structural Observations on the Stratum Corneum and Stratum Granulosum from Bovine Fore-Stomach. J. Dairy Sci. 50:997, 1967 (Abs.).

32. Lewis, A. E. Biostatistics. Reinhold Publishing Corporation, New York. 1966. Page 59.

33. Masson, M. J., and A. T. Phillipson. The Absorption of Acetate, Propionate, and Butyrate from the Rumen of Sheep. J. Physiol. 113:189, 1951.

34. Maynard, L. A., and J. K. Loosli. Animal Nutrition. Page 290. 4th Edition. McGraw-Hill Book Company, Inc. New York. 1956.

35. Murray, M. G., R. S. Reid, and T. M. Sutherland. The Rate of Passage of Digesta from the Reticulo-Rumen of Sheep. J. Physiol. 164:26p (Abs.), 1962.

36. Parthasarathy, D., and A. T. Phillipson. The Movement of Potassium, Sodium Chloride, and Water Across the Rumen Epithelium of Sheep. J. Physiol. 121:452, 1953.

37. Pennington, R. J. The Metabolism of Short Chain Fatty Acids in the Sheep. I. The Fatty Acid Utilization and Ketone Body Production by Rumen Epithelium and Other Tissues. Biochem. J. 51:251, 1952.

38. Pfander, W. H., and A. T. Phillipson. The Rates of Absorption of Acetic, Propionic, and N-Butyric Acids. J. Physiol. 122:102, 1953.

39. Senior, J. R. Intestinal Absorption of Fats. J. Lipid Res. 5:495, 1964.

40. Sisson, S., and J. D. Grossman. The Anatomy of Domestic Animals. Page 726. 4th Edition. W. B. Saunders Company, Philadelphia. 1953.

41. Smith, A. H., M. Kleiber, A. L. Black, and C. F. Blaxter. Transfer of Phosphate in the Digestive Tract. J. Nutr. 57:507, 1955.

42. Smyth, D. H., and C. B. Taylor. Intestinal Transfer of Short-Chain Fatty Acids In Vitro. J. Physiol. 141:73, 1958.

43a. Snedecor, G. W. Statistical Methods. 5th Edition. The Iowa State University Press, Ames. 1956. Page 87.

43b. Ibid., page 455.

44. Stacy, B. D., and A. H. Brook. The Renal Response of Sheep to Feeding. Aust. J. agric. Res. 15:289, 1964.

45. Stevens, C. E. Transport of Sodium and Chloride by the Isolated Rumen Epithelium. Am. J. Physiol. 205:1099, 1964.

46. Stevens, C. E., and B. K. Stettler. Factors Affecting the Transport of Volatile Fatty Acids Across Rumen Epithelium. Am. J. Physiol. 210:365, 1966.

47. Stevens, C. E., and B. K. Stettler. Transport of Fatty Acid Mixtures Across Rumen Epithelium. Am. J. Physiol. 211:264, 1966.

48. Stevens, C. E., and B. K. Stettler. Evidence for Active Transport of Acetate Across Bovine Rumen Epithelium. Am. J. Physiol. 213:1335, 1967.

49. Sutherland, T. M. in Progress in Nutrition and Allied Sciences. Page 159. Edited by D. P. Cutherbertson. Oliver and Boyd, Edinburgh. 1963.

50. Sutton, J. D., A. D. McGilliard, and N. L. Jacobson. Technique for Determining the Ability of the Reticulo-Rumen of Young Calves to Absorb Volatile Fatty Acids at Controlled pH. J. Dairy Sci. 45: 1357, 1962.

51. Tsuda, T. Studies on the Absorption from the Rumen. I. Surgical Operation of Forming the Miniature Rumen. Tohoku J. Agric. Res. 7:231, 1957.

52. Tsuda, T. Studies on the Absorption from the Rumen. II. Absorption of Several Organic Substances from the Miniature Rumen of the Goat. Tohoku J. Agric. Res. 7:241, 1957.

53. Tsuda, T. The Relationship Between Rumen Motility and the Rate of Acetic Acid Absorption from the Rumen by the Use of the Rumen Pouch Method. Tohoku J. Agric. Res. 13:29, 1962.

54. Tsuda, T. Absorption of Water from the Rumen by the Use of Rumen Pouch Method. Tohoku J. Agric. Res. 15:83, 1964.

55. Turner, A. W., and E. V. Hodgets. Buffer Systems in the Rumen of the Sheep. Aust. J. Agric. Res. 6:125, 1955.

56. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. Manometric Techniques. 4th Edition, 1964. Burgess Publishing Company, Minneapolis 15, Minnesota, page 132.

57. Ussing, H. H., and K. Zerhan. Active Transport of Sodium as the Source of Electric Current in the Short-Circuited, Isolated Frog Skin. Acta Physiol. Scand. 23:110, 1951.

58. Vidacs, G., G. M. Ward, and G. Nagy. In Vitro Studies of Volatile Fatty Acids Transfer Through the Rumen Epithelium Using a New Instrument. J. Dairy Sci. 45:691, 1962 (abs.).

59. Waldern, D. E., V. L. Johnson, and T. H. Blosser. Cardiac Output in the Bovine and Relationship to Rumen and Portal Volatile Fatty Acid Concentration. J. Dairy Sci. 46:327, 1963.

60. Warner, A. C. I., and B. D. Stacy. Solutes in the Rumen of the Sheep. Quart. J. Expl. Physiol. 50:169, 1965.

61. Werbin, H., I. L. Chaikoff, and M. R. Imada. Rapid Sensitive Method of Determining H^3-Water in Body Fluids by Liquid Scintillation Spectrometry. Proc. Soc. Expl. Biol. Med. 102:8, 1959.

62. Wilson, T. H. Intestinal Absorption. W. B. Saunders Company, Philadelphia. 1962.