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Sustained release delivery system for ruminant animals

Elaine L. Mahoney

To the Graduate Council:

I am submitting herewith a thesis written by Elaine L. Mahoney entitled "Sustained release delivery system for ruminant animals." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

J.W. Holloway, Major Professor

We have read this thesis and recommend its acceptance:

W.T. Butts Jr, C.C. Melton

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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SUSTAINED RELEASE DELIVERY SYSTEM FOR RUMINANT ANIMALS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Elaine L. Mahoney

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ABSTRACT

Ten in vivo trials were conducted to study the slow release delivery system. Trials 1, 2, 5, and 6 involved the use of one ruminally fistulated steer and were designed to study the effect of the following variables on the rate of release of slow release delivery system (SRDS), paper weight (Kg basis) within one paper type, paper weights among different paper types, and different covering types (coarse vs dense vs none) used to cover the exposed ends of SRDS(s). Twenty-four nonfistulated mature Angus, Herferd, and Angus-Herferd cows were used for trial 3 to investigate the effect of diet and animal variation on the rate of release of cromium oxide from SRDS. Fecal samples were collected every other day and analysed for cromium. Trial 4 involved the use of 24 nonfistulated heifers to study the release of Rumensin® from SRDS(s). Daily gain, hay consumption, and feed efficiency were recorded for each animal and compared with responses of control animals and animals receiving orally administered Rumensin®. Trials 7, 8, 9, and 10 were employed using six ruminally fistulated Jersey females. In trials 7 and 8, the effect of diet and animal variation among and within animals, on the rate of release of SRDS's (containing waterproof membranes) were studied. Trial 9 was conducted to evaluate consistency of release of Ciba-Geigy A-72662 (a systemic fly insecticide) from a SRDS. Fecal samples were collected from each animal on alternate days during the first

and last two weeks of the trial and once a week per animal during other weeks. Trial 10 was employed to measure the release of Ciba-Gelgy A-72662 from a modified SRDS design. Fecal samples were collected once a week per animal. Fecal samples from trials 9 and 10 will be analyzed for ovacidal properties.

Weight of paper (Kg basis) had a significant effect ($P<.001$) on rate of exposure of SRDS(s). There was a trend for thinner paper (13.6 Kg basis) to degrade at a faster rate than thicker paper (31.8 Kg basis). Moreover, there was a relatively large amount of variation in rate of exposure between different paper types and weights (Kg basis). Covering type also had a significant effect on rate of exposure of SRDS's ($P<.01$). The interaction of covering type and basis was highly significant ($P<.001$). Day also had a significant curvilinear effect on the rate of exposure of SRDS ($P>.0002$). Hence, the rate of paper membranes exposed per day decreased as the time SRDS(s) remained in the animal increased.

No cromium oxide was detected in the feces collected from animals in trial 3. However, Rumensin® was successfully released from SRDS(s). Daily gain for animals receiving Rumensin® via SRDS was similar to heifers fed Rumesin® in supplement (.87 Kg/day vs .86 Kg/day). Rumensin® treated groups (either by SRDS or by supplement) had increased gains within the range experienced by other workers. Rumensin® treated heifers were also more efficient than heifers who received no Rumensin® (4.88, 3.70, and 5.59 pound hay/pound gain).

Diet (fescue-legume vs fescue) had no effect on the rate of exposure of SRDS ($P>.1$). Additionally, there was no variation among and within animals on the rate of exposure of SRDS(s) ($P>.1$).

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CHAPTER I

INTRODUCTION

Currently, several chemical compounds are on the market which exhibit a beneficial effect upon ruminant animals. Several compounds are known which improve feed efficiency. Other compounds are useful in promoting growth and improving the yield of meat utilized for human consumption. Additionally, some pharmaceuticals are effective in the therapeutic treatment and prophylastic control of ruminant diseases such as parasite infestation. Markers used for estimation of fecal output, forage intake and digestibility are also being investigated. Several of these useful agents are effective only when administered via the rumen of the animal. Consequently, these drugs are commonly administered by daily dosing in the form of feed additives, licks, water additives and the like. It is essentially impossible to administer drugs which require daily dosing by feed additives or the like because some animals are range fed over long periods of time. Moreover, daily administration of drugs via injection to feedlot animals is uneconomical.

Recently, efforts have been made to develop an economical drug delivery device which would release to the stomach of a ruminant a predetermined amount of pharmacological agent at a controlled and continuous rate for a prolonged period of time. Many problems have been encountered in the development of effective means for

economically administering such controlled release formulations to the rumen of domesticated ruminant animals. The purpose of this study was to develop a slow release delivery system which is (1) economical, (2) easily administered, (3) remains in the reticulo-rumen for a predetermined amount of time, and (4) safe, in that it will not permit excessive, lethal amounts of formulated drug to be administered to the animal.

CHAPTER II

REVIEW OF LITERATURE

1. Types of Sustained Release Delivery Systems

Diffusion Mechanism

This type of slow release delivery system consists of a three layer laminate comprising a core lamina of a crystalline drug of low water solubility dispersed homogeneously in a polymer matrix with permeability to the drug. Hence, this particular delivery system dispenses active agents by a diffusion mechanism (Higuchi et al, 1978). Initially, the drug molecules dissolve in the matrix material. Subsequently, the core laminae, which have a specified thickness and exposed surface areas, releases the active agent. The active agent permeates either through exposed surface areas or to outer laminae (rate controlling laminae). A relatively constant release rate may be achieved provided there is a particular correlation between the respective permeabilities, thicknesses and exposed surface areas of the core laminae and the outer laminae.

Mechanical Mechanism

The mode of release of another type of slow release delivery system is initiated by a mechanical mechanism (Laby, 1974). Specifically, the device is comprised of a cylinder shaped body

portion which is structured in a way to be adopted to oral administration to the ruminant animal. The first configuration may be retained via a band of soluble material such as geletin tape. Once in the rumen, the device is changed into a second configuration (an expanded configuration) which is aimed to prevent or hinder regurgitation of the device. Alternatively, a spring load device equipped with expandable plastic strips may also be effective in retaining the device in the rumen following oral administration. Thus, the obstructing mechanism may be composed of one or more flaps, tabs, flanges, loops, bubbles, blisters, or like protuberances. Once the device has opened into the second configuration, contents of the rumen can make contact with the material contained in the semi-cylinders. The body portion of the device may be comprised of a relatively insoluble, resilient matrix material which contains a material to be released in the rumen. The material within the slow release delivery system may be retained in the matrix material in the form of a suspension, a solid solution or it may be weakly chemically bonded to the matrix material. Release of the material within the slow release delivery system, over an extended period of time, may be accomplished in the following ways: The material may be enclosed in a number of capsules which have different solubilities in rumen fluid; tablets of the material may be coated with other substances which have different solubilities in rumen fluid; container portions incorporated into the device may be permeable in whole or in part to the material of the rumen fluid.

Biodegradation and Erosion

Another type of slow release delivery system is constructed using a metal cylinder which is open at both ends. The cylinder is equipped with a retaining means within the cylinder which functions to retain the formulated pharmacological agent within the cylinder. The drug retaining means consists of a series of circular grooves cut into the inner wall of the steel cylinder. The active ingredient within the cylinder is uniformly admixed with a copoly matrix which is subsequently released via biodegradation and erosion of the formulation when exposed to the stomach fluids of a ruminant (Simpson, 1980). This slow release delivery system design provides a constant surface area of exposed formulation to ensure uniform and constant delivery of the drug. After oral administration of the slow release delivery system, this device is of such a weight that it remains in position in the reticulo-rumen portion of the ruminant stomach, not only when filled with formulation, but also following total discharge of the drug.

Dispersion Mechanism

Still another type of slow release delivery system designed to release pharmaceutical agents is the dispersion mechanism (Goldberg and Franklin, 1979). This slow release delivery system is constructed using a stack of edible paper webs which are fabricated into a unit dosage by laminating only the edges of the paper. Upon ingestion, the laminated edges rupture which enables the stack of

webs to separate and disperse. Once dispersion occurs, finely particulate medicament loaded to at lease one of the sheets of web becomes exposed for absorption. Two different mechanisms for controlling the rate of release of medicament from edge seal laminated unit dosage forms are employed for this particular slow release delivery system. The first of these is the use of improved carboxymethylcellulose paper sheets used solely as a means to separate the medicament loaded sheets from the body of the edge seal laminates. In this instance, the pharmaceutical is loaded onto sheets of conventional paper. In the stomach, the sheets of paper function to delaminate the unit dosage form. The second method utilizes the paper sheets both as delaminating and disintegrating means. The medicament is loaded to the improved paper composition sheets; i.e., the sheet serves as a substrate for the medicament.

Mechanism for Adjusting Surface Area

Lastly, another type of slow release delivery system consists of a cylindrical sintered polythene outer shell impregnated with cellulose acetate. The rate of release is controlled by adjusting the surface area of the membrane to ruminal fluid by means of an inner stainless steel sleeve which contains holes of finite number and dimension (Jones, 1981). The specific gravity of the slow release delivery system, regulated by the thickness of the inner stainless steel sleeve, provides adequate retention in the reticulorumen for the projected active life of the slow release delivery system.

One of the primary purposes of the experiments reported herein was to determine the rate of release of a slow release delivery system, constructed with sequentially degradable paper membranes, in the reticulo-rumen. Cellulose is assumed to be a major component of paper and is readily digestible (Hensen et al, 1969). Hence, factors affecting the breakdown of cellulose such as cellulolytic bacteria, nutrient requirements of the microbes, and the like, may be feasibly applicable to degradation of paper.

2. Factors Affecting Cellulose Digestion

Cellulolytic Bacteria

Utilization of cellulosic materials by the ruminant is dependent upon certain species of anaerobic bacteria, and to a lesser extent, ciliate protozoa which inhabit the rumen. Cellulose enzymes, which function to hydrolyze the insoluble cellulose to soluble cellulodextrines and/or sugars, are produced by these bacterial species (Shafer and King, 1965; Sheth and Alexander, 1969). Subsequently, hydrolyzed products may then be absorbed and fermented to obtain energy required for growth. Final products of cellulose fermentation (acetate, propionate, butyrate, carbon dioxide, methane and microbial cells) are not produced by cellulolytic species alone. There is a complex interaction among populations of (1) cellulolytic species, (2) other carbohydrate fermenting species which ferment soluble carbohydrates metabolized from the former cellulolytic species, (3) species that degrade succinate and formate, and in

some conditions, lactate produced by carbohydrate fermenting species, and (4) methanogenic bacteria which reduce carbon dioxide to methane by utilizing hydrogen gas or formate (electron donnors) produced by other species in order to obtain energy for growth (Hungate, 1966; Hungate et al, 1970).

According to Slyter et al (1971) cellulose producing bacteria represent approximately one quarter or less of the viable bacteria even when cellulose is the only energy source in the diet.

Species responsible for degrading cellulose (at least their relative numbers and the abilities of pure cultures to attack various forms of cellulose) have been reported. The most important species include Bacteroides succinogenes (Hungate, 1950), Ruminococcus flavefaciens (Sijpesteijn, 1951), Butyrivibrio fibrisolvens (Bryant and Small, 1956), and Ruminococcus albus (Hungate, 1957). Other species, including the genus Clostridium and Cilobacterium Cellulosolvens (Bryant and Small, 1956; Hungate, 1957; Shane et al, 1969 and Van Gylswyk and Hoffman, 1970) have also been found on occasion. However, these strains have less apparent contribution to cellulose degradation.

Evidently, carbohydrate fermentation is the only method of obtaining chemical energy for the four major cellulolytic species. With the possible exception of some strains of Butyrivibrio fibrisolvens which ferments some amino acids, other substrates such as fatty acids, lactate, and glycerol are not fermented by cellulolytic species. Ruminococci primarily ferment cellulose and pentosans,

whereas Bacteroides succinogenes ferment cellulose and starch (Akin, 1979). Chen et al (1969) reported Butyrivibrio fibrisolvens is one of the most versatile bacteria in the rumen, fermenting a large number of carbohydrates and carbohydrates containing compounds such as saponins and rutin. Bryant and Small (1956), Shane et al (1969), and Van Glysloyk and Roché (1970) found, however, that most strains of Butyrivibrio fibrisolvens do not ferment cellulose.

Data from several sources (Halliwell and Bryant, 1963; Dehority et al, 1967; Kock and Kistner, 1969) indicate Bacteroides succinogenes to be the most active cellulosic organism in the rumen. Additionally, this particular strain digests the more resistant cellulose (cotton fibers and mature hay) to a greater magnitude than other species. The Ruminococci are also quite active, yet they show inconsistency among strains in their ability to degrade the more resistant cellulose. Butyrivibrio fibrisolvens strains, on the other hand, are relatively inactive in degrading cellulosic material when compared to Bacteroides succinogenes and Butyrivibrio fibrisolvens. A study conducted by Van Gylswyk and Labuschagne (1971) on the rate of digestion of web ball-milled Whafman No. 1 fiber paper confirmed the greater cellulolytic activity of Ruminococci as compared to Butyrivibrio fibrisolvens, and revealed that Ruminococcus albus digested this form of cellulose more rapidly then Ruminococcus flavefaciens.

Nutrient Requirements

One or more of the B-vitamins are required for the growth of all the bacterial strains thus far studied. Bryant et al (1959) and Scott and Dehority (1965) stated that strains of Bacteroides succinogenes always require biotin for growth with p-aminobenzoic acid being stimulatory to some. In addition to biotin, most strains of Ruminococcus albus requires pyridoxine, and are stimulated to grow by p-aminobenzoic acid (Bryant and Robinson, 1961a; Scott and Dehority, 1965). These studies also indicated the requirement of folic acid, riboflavin and thiamin by a few strains of R. albus. Further, vitamin requirements of R. flavefaciens are similar to those of R. albus except that fewer strains of R. flavefaciens need pyridoxine for growth. Some strains of R. flavefaciens require colbalamin and, in some instances, methionine may be substituted in its place (Scott and Dehority, 1965).

Information concerning mineral requirements of cellulolytic bacteria is scarce, but it may be reasonable to assume their essentials are similar to other bacteria. However, contrary to previous studies of most nonmarine bacteria (Macleod, 1965), Bryant et al (1959) found Bacteroides succinogenes has a requirement for sodium and an obligatory amount of calcium. Work with mixed cultures has shown that potassium, sodium, calcium and phosphate were necessary for optimum cellulose digestion.

Detailed studies of sulfur requirements for cellulosic bacteria have not been extrapolated. Simplistically, however, Bryant et al

(1959) found B. succinogenes utilize either cysteine or sulfide, but not sulfate. Allison et al (1958) found sulfide to be a probable essentiality for some strains of R. flavefaciens. He also observed R. albus and R. flavefaciens to grow well in media containing only sulfide and sulfate sulfur. The study of Emery et al, (1957) suggested that B. fibrisolvens utilized sulfate sulfur whereas B. succinogenes and Ruminococci did not.

In general, ammonia is essential as the main nitrogen source for Ruminococci and B. succinogenes and is utilized as the sole nitrogen source by most strains (Bryant et al, 1959; Bryant and Robinson, 1961b; Dehority, 1963). Allison et al (1959) and Bryant and Robinson (1963) suggested that these microbes were ineffective in utilizing organic nitrogen and amino acid carbon. The reasons for the inability of these bacteria to use organic nitrogen sources for growth are obscure. It is possible, however, that these microbes lack mechanisms to transport materials, such as most amino acids or peptides, into the cell (Pittman et al, 1967).

Gill and King (1958), Bryant and Robinson (1962) and Shane et al (1969) suggested that strains of B. fibrisolvens may be divided into two main groups based on nitrogen requirements. One group depends on ammonia for growth while the other relies on amino acids for survival. No single amino acid was essential for growth; and, interestingly, a large amount of ammonia was assimilated regardless of the amino acid content of the medium (Gill and King, 1958).

Carbon sources, other than those used as the energy source, are required for growth by strains of cellulolytic bacteria. Dehority (1971) summarized the requirements for carbon dioxide and bicarbonate as follows: B. succinogenes and R. flavefaciens have an essential requirement for large amounts of these compounds for optimal growth since they fix carbon dioxide into pyruvate in the pathway to succinate the main repository for removal of electrons generated during glyclysis (White et. al 1962; Caldwell et al, 1969). Carbon dioxide may also be used for biosynthetic purposes by the organisms (Allison, 1969 and 1970). B. fibrisolvens and R. albus may or may not need small amounts of carbon dioxide for initiation of growth, however, small amounts are needed for optimal growth (Dehority, 1971). Presumably, these species require carbon dioxide primarily for biosynthetic purposes.

Most strains of B. succinogenes, R. flavenfaciens and R. albus also require volitile fatty acids for growth. Data from Bryant and Doestch (1954), Allison et al (1958 and 1962), Hungate (1963), Wegner and Foster (1963) and Dehority et al (1967) observed the following volitile fatty acids essential for growth: In general, B. succinogenes require valeric, 2-methylbutyric or isobutyric. R. flavefaciens and R. albus require isovaleric, 2-methylbutyric and isobutyric. It should be noted that some variation does exist for essential volitile fatty acids requirements among strains within the same species.

Cellulolytic, as well as noncellulolytic species employ one or more of the acids, isobuyric, isovaleric, or 2-methylbutyric for biosynthesis of the amino acids, valine, leucine, or isoleucine, respectively (Allison et al, 1959; Allison, 1962, 1969 and 1970; Robinson and Allison, 1969; Kunsman, 1970) via reductive carbodylation reactions (Allison and Peel, 1971).

The essentialness of these acids by the cellulolytic bacteria stresses the complex interactive system among rumen bacterial species. The n-valerate is formed either from carbohydrate or from amino acids such as proline (Elsden, 1956 and Dehority et al, 1958). In contrast, Allison (1970) reported the branched chain acids, isoburyric, isovaleric and 2-methylburyric are primarily dervied by oxidative deamination and decarboxylation of the amino acids, valine, leucine and isoleucine, respectively. Hence, formation of branch chain acids and ammonia by some strains of noncellulolytic bacteria, B. ruminicola and Megasphaera elsdenii (Allison, 1970) are needed by cellulolytic bacteria. In turn, the noncellulolytic species probably require soluble carbohydrate products of the cellulolytic bacteria as energy yielding substrates, particularly when diets are fed that contain cellulose as the main carbohydrate source. Coleman (1967) detected branched chain acid formation by ciliate protozoa. According to Slyter and Weaver (1971), dietary amino acids or protein are not necessary for branched chain volitile acid biosynthesis in the rumen. In the presence of cellulose as the main energy source, mixed rumen bacteria, either in the rumen

or in the continuous flow fermentor, were still capable of synthesizing branch chain volitle acids regardless of the absence of dietary amino acids. In addition, cellulolytic bacteria isolated under such conditions also require either one or more of the branched chain acids. In some instances, valeric acid was also needed for growth. One feasible explanation for this observation is that some of the bacteria capable of biosynthesizing the amino acids from carbohydrate and carbon dioxide carbon are lysed with subsequent degradation of the protein and amino acids in the same manner as dietary constituents.

Emphatically, when purified low quality diets high in cellulose, and lacking in some of the essential nutrients needed by cellulolytic bacteria are fed to ruminants, cellulolytic species continue to grow and maintain their function, at least to a limited extent because of the production of these requirements by other bacteria.

Mode of Microbial Attach On Plant Tissues

The use of microscopic techniques has enhanced the understanding of factors affecting forage degradation by rumen microorganisms. A review by Akin (1979) on microscopic evaluation of forage digestion by rumen microorganisms stated there were differences in plant anatomy and sites of lignification that affected the digestibility among species and plant parts. Moreover, variations exist

in the ease and mode of digestion of similar tissue types among grass types, species and cultivars.

An experiment on progressive digestion of bermuda grass and Kentucky-31 tall fescue revealed that even though leaf tissues of different forage grasses vary in arrangement, amount and cell wall thickness (Brown, 1958; Metcalfe, 1960), the general pattern for ease and extent of tissue digestion by rumen microorganisms was as follows: Mesophyll, Phloem $\geq$ Epidermis, Parenchyma bundle sheath $\geq$ Sclerenchyma $\geq$ lignified vascular tissue (Akin and Burdick, 1975). This pattern with slight variations has been observed by other studies conducted with a light microscope (Drapala et al, 1947; Regal, 1960; Hanna et al, 1973) and with a scanning electron microscope (Harbers and Thouvenelle, 1980).

Although this sequence of degradation by rumen microorganisms is generally accepted, work done by Akin and Burdick (1975) showed that similar tissues in different forage, or even in cultivars within a species, are degraded at different rates. Apparently, inherent cell wall characteristics (e.g., composition, chemical linkages) influence cell wall digestibility. Hanna et al (1973) and Akin and Burdick (1975) observed variations in rates of digestion of cell walls within and among plant species.

It is generally accepted that an increase in plant maturity and hence, an increase in lignification is associated with a decrease in digestibility. Akin et al (1977) found that decreased digestibility with increased maturity was correlated with a specific type

of lignin known as syringyl lignin. Pigden (1953) related increases in lignification and amounts of lignified cells decreased cellulose digestion as well as decreased digestion of the more soluble nutrients within the cells.

In reviewing the mode of attack by cellulolytic species, Hungate (1966) showed that pure clutures of some cellulolytic rumen bacteria move to a position adjacent to their substrate to derive nutrients from cellulose digestion. Viewing this observation with a transmission electron microscope confirmed that rumen bacteria do degrade readily digestible tissues (e.g., mesophyll, phloem) by close proximity to plant walls but without direct adherence. However, the more rigid and slowly digestible tissues (e.g., the epidermis and parenchyma bundle sheath) require direct attachment by rumen bacteria prior to digestion (Akin et al, 1974). Van Soest (1982) reported digestion of crystalline cellulose occurs only in the attached phase.

Two types of bacteria which predominate in the rumen adhere to and degrade to the slowly digestible plant walls in the initial stages. These are (1) an encapsulated coccus resembling Ruminococcus (Buchanan and Gibbons, 1974; Patterson et al, 1975; Latham et al, 1978) and (2) a lucid irregular shaped bacterium resembling B. succinogenes (Buchanan and Gibbons, 1974; Costerton et al, 1974). During degradation, these two types of microbes adhere to forage walls in closer proximity than other types (i.e., regular shaped rods and cocci). The latter types are less frequently

associated with degradation zones (Akin, 1976). According to Van Soest (1982), cell wall digesting organism accomplish attachment by etching pits into available surfaces. Evidently, the extra cellular material of the encapsulated coccus initiated bacterial adhesion to the plant cell walls. In contrast, the irregular shaped bacterium adhere to the substrate without formation of a distinct capsule but with subsequent conformity of bacterium shape to the shape of the eroded zone (Akin, 1976). Apparently, each bacterial type can degrade entire cell walls via enzymatic action alone (Akin and Amos, 1975). Further, Leatherwood (1973) stated that R. albus may have an "affinity factor" which may be necessary to hold the "hydrolytic factor" in position to the insoluble cellulose in order for multiple attacks to occur.

Effect of Diet on Number and Kinds of Microorganisms

Variability in the components of feed is one of the main factors affecting rumen fermantation (Hungate, 1966). Bryant and Burkey (1952) indicated there are fluctuations in the numbers and kinds of bacterial flora of cows and sheep when different rations are fed. Gall et al (1949) cultured bacteria from the rumen of cattle and sheep on practical farm rations and found a rather uniform distribution of the kinds of bacteria present except the numbers of fast growing organisms increased with the amount of grain in the ration. In a similar study, differences were seen in the number and kinds of bacteria present in the rumen of sheep on

purified diets containing urea-plus sulfur, urea without sulfur and casein (Gall et al, 1951).

Another study was conducted by Slyter et al (1968) to observe the kinds of ureolytic bacterial species present when animals were fed either natural or purified diets. Results from this study revealed the percentage of facultative anaerobic and H_2S producing ruminal bacteria were greater for steers fed biruet than for steers fed other NPN sources. Steers fed uric acid had more cellulolytic bacteria than steers fed urea and urea phosphate. Steers fed urea had more amylotytic bacteria than steers fed urea phosphate. Additionally, cattle fed a 30% roughage--70% concentrate natural diet contained more ruminal protozoa and gram-negative rod shaped bacteria, but had fewer cocci than cattle fed urea or isolated soy supplemented purified diets.

Bacteria cultures established from rumen contents of steers fed corncob alfalfa hay rations with and without added starch and with varying amounts of casein revealed variations in both the kinds and numbers of bacteria present (Burroughs et al, 1950). Hungate et al (1952) found the ruminal flora of sheep changed rapidly when large amounts of grain or glucose were placed in the rumens of these animals that had been maintained on a ration of alfalfa hay. Specifically, the numbers of strepto coccus bovis increased while the numbers of other kinds of bacteria decreased.

Results from a series of experiments conducted by Bryant and Burkey (1952) revealed there was very little difference in the numbers of bacteria found on rations other than concentrate. Further, when concentrate mixture alone was fed, the numbers of bacteria cultured were higher and became progressively more numerous with the length of time the animal was on the ration. Specifically, this study also showed that cellulolytic cocci were a relatively constant percentage of the total isolates from the different experiment. In contrast, cellulolytic rods varied from no isolations on the concentrate ration to 20.4% of the bacteria isolated on the straw ration. Interestingly, animals fed the concentrate diet maintained cellulolytic cocci in high numbers even though the ration contained only 6.3% fiber.

3. Kinetics of Digestion

The relationship of rate and extent of digestion with respect to forage utilization by the ruminant involves very complex interactions among plant components, microorganisms in the rumen and the animal.

Ingested feed and water may leave the digestive tract via two major processes. These are: (1) breakdown and absorption due to digestion and (2) explusion due to passage (Van Soest, 1982). According to Mertens and Ely (1982), digestion and passage can be further divided into phases. Basically, the digestion process may by subdivided into two stages, microbial interaction and attachment

and chemical breakdown. Passage may be divided into particle size reduction, escape from the rumen and movement of particles through the tract. The processes of digestion and passage will first be discussed as individual entities with subsequent discussion of the two processes in unification.

Mathematical models have been employed in the attempt to better understand these multiple relationships of digestion (Balch and Campling, 1965; Waldo, 1969; Gill et al, 1969; Mertens, 1977) and others (Smith et al, 1972; Tauskey et al, 1972; Baldwin et al, 1977 and Mertens and Ely, 1982).

Factors Affecting Digestion Parameters

Regardless of the model chosen to describe the dynamic digestive process, Mertens and Ely (1982) acknowledged three components, which may vary among forages, that affect digestion parameters: Size of the potentially digestible fractions, rates of digestion and digestion lag.

Potential Extent of Digestion

Of these three components, Mertens (1977) and Mertens and Ely (1978a,b) found the potentially digestible fraction to have the largest effect on the digestibility of forages. Chemical composition (Van Soest and Jones, 1968; Smith et al, 1972 and Denium, 1973), plant morphology (Akin et al, 1974 and Akin and Amos, 1975), and possibly crystallinity (Tomlin, 1960 and Caufield and Moore, 1974)

may affect this potential extent of digestion. According to Smith et al (1972), chemical composition of forages has the greatest impact on this component of the digestion model. Results from their work showed correlations of .78 or higher between lignin and the 72 hour cell wall digestibility used to predict the inherent digestible fraction. More research is needed to define the fraction that is truly indigestible. Abrams and Moore (1979) indicated that the "indigestible fraction" measured by in vitro and in situ methods is digestible implying that the "indigestible fractions" are not indigestible, but have a very slow rate of digestion. Unfortunately, current biological and chemical methods do not determine or isolate the "indigestible fraction", hence, mathematical estimates, using double reciprocal plots of indigestible residue versus time (Mertens and Van Soest, 1972) could perhaps be employed to characterize its magnitude.

Rate of Digestion

As previously stated, plant, microbal and animal factors could be responsible for variations in fractional rates of digestion. Smith et al (1972) and Mertens (1973) reported that forages differ in rate of neutral detergent fiber digestion. Hence, hypothetically, intrinsic characteristics of the plant sets the upper limit for rate of digestion. Smith et al (1972) also found measured chemical composition to be inconsistently related to rate of digestion. From this observation, it may be concluded that chemical composition does

not significantly affect the rate of digestion, or more realistically, discrepancies in present chemical and physical methods do not accurately measure the characteristics responsible for controlling digestion rate. Baldwin et al (1977) stated that the inability to isolate chemical components in their native states and the overall complexity of ruminant digestion have hindered progress in studying rate of digestion.

Additionally, present methods for mathematically describing digestion rates may represent artifact rates that are a function of the model used. For instance, if the single digestible fraction used by Smith et al (1972) was in actuality an aggregation of several digestible fractions, then the single digestion rate was confounded with average individual rates. Hence, the hypothesis of single rates being associated with a particular chemical constituent is still possible. The rate of digestion varies among morphological tissue types (Akin et al, 1974; Akin and Amos, 1975). Furthermore, morphological types of leaf tissue contain various proportions of cellulose, hemicelluloses, pectins and lignins, which in themselves vary in rate of digestion. Hence, difficulty of relating chemical constituents to a single rate is amplified.

It would seem reasonable to postulate that those factors which influence growth and reproduction of microbes, or alter the makeup of the ruminal microbial population influence would also affect the rate of digestion. Unfortunately, few studies have been employed in studying correlations between microbial populations and rate of

digestion. In vitro studies conducted by El-Shazly et al (1960); Little et al (1970) and Martinez and Church (1970) revealed that limiting the amount of nitrogen, minerals and trace compounds resulted in reduced extent of fiber digestibility. However, it is not certain whether or not this reduction is due to changes in rate of digestion. It is possible that soluble carbohydrate-digesting microbes utilize limited nutrient supplies before fiber digesting bacteria can increase in number. In this theoretical situation, it seems likely that fiber digesting enzymes would not be produced in concentrations high enough to render optimum rates of fiber digestion. Further, Harrison et al (1976) and Chalupa (1977) proposed that changing rumen turnover alters the composition of the microbial population. If fiber-digesting bacteria grow more slowly than other types, then faster rumen liquid turnover may reduce their proportion in the total microbial population. Subsequently, rate of fiber digestion may decrease. Rate of fiber digestion may also be reduced by low pH in the rumen. According to Slyter et al (1966) and Russell et al (1979), pH affects the ration of microbial species and enzymatic activity. Stewart (1977) indicated that cellulose activity is inhibited by pH below 6.0.

The last factor to consider which can alter the fermentation environment, thereby influencing the rate of digestion, is the animal itself. Mastication with subsequent rumination of feed stuffs results in a reduction of particle size. In turn, particle

size reduction causes an increase surface area accessible for enzymatic attack.

Work done by Dehority and Johnson (1961) and Troelsen and Bell (1969) indicated that reducing particle size via grinding increases *in vitro* digestibility. In these experiments grinding was not extensive enough to affect the molecular relationship between lignin and fibrous carbohydrate. Hence, it can be assumed that the effect of grinding increased rate of digestion due to a decrease in particle size rather than decreasing the digestible fiber fraction. Continuous *in vitro* studies conducted by Robles et al (1980) showed increased rates of digestion when particle size of some forages was reduced. Conversely, Brazle and Harbers (1977) and Cheng et al (1979) indicated an increase in microbial attack of fibers when the epidermal layer of plant tissue is fractured although tissues were not ground to small particle size. Terry et al (1972) and Osbourn et al (1974) suggest that other factors such as plane of nutrition, water soluble carbohydrate concentration, or buffering capacity may be more important than particle size in explaining variations between *in vitro* and *in vivo* cell wall digestibility.

Digestion Lag

Several factors may affect this initial stage of digestion. Mertens (1973) found forages differ in discrete lag time when inoculated with the same source of rumen fluid and incubated under the same conditions, hence, forage substrates must differ according

to hydration or rate of chemical or physical alteration prior to enzymatic degradation. Plant morphology may contribute to this complex phenomena (Brazle and Harbers, 1977). Mertens and Ely (1982) revealed the possibility that chemical or physical inhibitors must be removed or that swelling by hydration is necessary before enzymes can contact of and react with fiber molecules.

Microbial factors may also affect the lag phase. Specifically, if microbial species differ in rate or extent of attachment to forages (Akin, 1979; Van Soest, 1982) then factors which affect microbial populations could also alter lag time of digestion. The lag phase may also be partially due to the result of time required for bacteria numbers and fiber digesting enzymes to reach nonlimiting concentration levels (Mertens and Ely, 1982).

Finally, nonfiber components of the diet may contribute to lag time. If microbes have a preference for starch and other fermentable carbohydrates, fiber digestion would be delayed and lag time would increase. An experiment conducted by Mertens and Loften (1980) showed the addition of starch to forages increased fiber digestion lag time.

Passage Processes

Mertens and Ely (1982) implied that the actual event of digestion is related to the time feed remains in the gastrointestinal tract. (Figure 1)

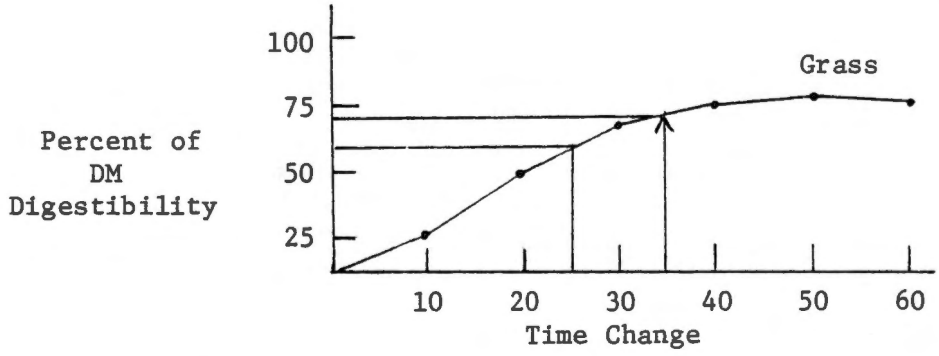

Figure 1. Effect of Change in Time Allowed for Digestion (Retention Time on Actual Extent of Digestion).

Time allowed for digestion is a function of rate of passage, therefore, the process of passage must be explained in terms related to the physiology of the animal yet be manipulated mathematically to estimate digestibility. A dynamic model evaluation of the relationship of rate and extent of digestion to forage utilization has been developed by Mertens and Ely (1982).

Factors Affecting Rate of Passage

Four main factors affect the perceived rate of passage: (1) Type of marker used, (2) daily intake, (3) physical form of the diet, and (4) rumination differences among animals.

According to Hungate (1966), soluble or small particle size markers such as polyethylene glycol, Cr EOTA or Cr_2O_3 pass out of the rumen at a slower rate than fiber. Smith (1968) explained this finding by stating that large lignified particles are selectively retained in the rumen. Hence, the quality of lignin may be related to the minimum passage rate of large particles, whereas liquid markers may represent the passage rate of small particles. Recently, Hartnell and Satter (1979a) have used several markers which are covalently attached to or have large absorption affinities for fiber. Although care must be taken to minimize movement of these markers between fiber particles, they appear to provide the technique to accurately measured movement of particulate matter through the gastrointestinal tract.

Additionally, several characteristics of the diet and the animal affect the rate of passage. Several studies experimenting with reduction of particle size via grinding have shown that changing the physical form of the diet in this way results in decreased retention time and increased rate of passage (Moore, 1964; Alwash and Thomas, 1971). There is also substantial evidence that retention time increases as feed intake decreases (Balch and Campling, 1965; Grovum and Williams, 1977). Thus, differences in individual daily feed consumption may, in part, contribute to differences in rate of passage among animals. Nonetheless, it is uncertain whether the increases in rate of passage associated with increased intake are due to rate of particle size reduction, rate in escape from

the rumen, or rate of passage through the intestines. It is doubtful that rate of particle size reduction increases as intake increases because the rumination time per kilogram of dry matter or cell wall consumed decreased as intake increases (Welch and Smith, 1969a; Sudweeks et al, 1980). Van Soest (1966) observed that fecal particle size increases as feed intake increases and suggested there are fluctuations in rate of escape from the rumen.

Hartnell and Satter (1979b) reported passage rates varied among animals fed similar diets suggesting individual animal characteristics influence passage rates. Variation in rate of passage among animals may be due to differences in rumination activity (Welch and Smith, 1969b, 1970, 1978). Cattle ruminate less per kilogram of dry matter than do sheep. Hence, rate of comminution may vary between species as well. Correspondingly, young, small animals of a species chew more per kilogram than older, large animals. Welch and Smith (1978), however, attribute this to the finding that smaller animals pass smaller fecal particle sizes. Little difference in overall gastrointestinal retention time results because increase rate of particle size reduction in small animals is counteracted by the rate of escape of large particle sizes which is essentially zero.

Integration of Digestion and Passage

The actual extent of digestion, or digestibility, is the outcome of competition between digestion and passage for each feed

particle. Because digestibility is the fraction of total disappearance (resulting from both digestion and passage) that can be ascribed to digestion, it can be calculated from the rates of digestion as depicted by Waldo et al (1972). Moreover, the model developed by Mertens and Ely (1979) can be used to estimate the effects of changes in rates of digestion and passage upon digestibility and maximum feed intake.

4. Utilization of Nonconventional Feedstuffs by Ruminant Animals

Feeding nonconventional substances to domesticated ruminants is becoming increasingly popular for several reasons: Due to an expanding human population and an increasing cost and demand for common animal feeds (e.g., corn and wheat), there has been an increasing public concern for reducing the expense of feeding animals used for human consumption. Specifically, ruminants, particularly cattle, sheep and goats, have the ability to utilize cellulose (Hansen et al, 1969) which is a major component of paper.

Feeding paper to ruminants is not a new technology. Mead and Gross (1935) added paper pulp to the diet of cattle and observed an increase in crude fiber digestibility. In another study, ground newspaper and magazines were added to diets as an absorbent carrier of a dry molasses product (Kesler et al, 1967). The feeding of other wood by-products, such as sawdust (Arichibald, 1926), ground mesquite wood (Marion, 1957) and wood pulp fines (Block and

Shellenberger, 1980) are also known to the art. Most of the studies reviewed herein were conducted to determine digestibility, palatability, and nutritive value of these residues with respect to the levels at which they could be efficiently utilized as roughage sources by ruminant animals (Daniels et al, 1970; Dinius and Oltjen, 1972; Millet et al, 1974; Dinius and Bond, 1975).

Large variations exist in the feeding values of various papers. Mertens et al (1971) and Van Soest and Robertson (1976) found in vitro digestibilities vary considerably among types of paper. This is in agreement with in vivo studies conducted by Mertens et al (1971), Dinius and Oltjen (1972) and Martz et al (1976).

Digestion characteristics, such as the time for feed to digest, lag, rate of digestion, amount of indigestible residue and amount of digestible fiber are important parameters in evaluation of paper as a feed source. Information concerning digestion characteristics of paper is limited. Recently, however, an in vitro study was conducted to study digestion characteristics of eight different paper types (Belyea et al, 1979). Results from this study indicated detergent fiber, insoluble ash and nitrogen content of papers had values similar to those reported by Van Soest and Robertson (1976). (Table 1).

Table 1. In Vitro Digestion Characteristics of Paper Detergent Fiber and Nitrogen Content of Waste Papers

Paper	NDF[a]	ADF[b]	ADL[c]	Cellulose	Ash[d]	Nitrogen
	(g/100 g of dry matter)					
Virgin corrug. unwaxed	96.52	83.40	13.86	69.54	2.8	.36
Virgin corrug. waxed	92.09	78.25	12.77	65.48	3.9	.41
Recycled corrug. unwaxed	95.66	82.83	11.00	71.83	2.0	.35
Recycled corrug. waxed	94.96	82.91	13.57	69.34	4.3	.34
Newsprint	98.02	77.69	22.17	55.52	6.7	.38
Office bond	90.08	84.05	3.30	80.72	10.2	.38
Solka floc	98.42	84.28	3.57	80.71	7.1	.33
Paper bags	97.63	87.72	9.06	78.66	1.5	.39

[a]Neutral detergent fiber (Goering and Van Soest, 1970).

[b]Acid detergent fiber (Goering and Van Soest, 1970).

[c]Acid detergent lignin ($KMnO_4$ option, Goering and Van Soest, 1970).

[d]Insoluble ash following ADL determination.

All papers contained larger amounts of neutral detergent fiber than most forages. Acid detergent fiber content was similar among the papers and was also considerably greater in magnitude than usually observed for forages. Variation in acid detergent lignin was observed among papers. The extent of delignification may account for this variation. Cellulose content of papers also varied primarily because of differences in lignin. In general, lignin content of the papers did not differ greatly from values typical of forage

(5% to 10% for grasses and 10% to 15% for legumes). According to Robles (1977) lignin to acid detergent fiber rations (.3910 to .2854) in the papers were similar to legumes (.3376) and greater than in grasses (.0683). Nitrogen and isoluble ash content were low in most papers and apparently, were not important factors in digestibility.

These papers were higher in cell wall than typically seen in legumes (40% to 60% NDF) and in grass (50% to 70% NDF). Specifically, office bond, solka floc and paper bags had small indigestible cell wall residues (8.04% to 16.57%), large amounts of potentially digestible cell wall (81.86% to 88.16%) and large digestion constants (.0450 to .0540). Unwaxed corrugated papers (heavy papers with alternating ridges and grooves) had moderate potential digestibe cell wall (68.93% and 69.56%) and moderate to high rate constants (.0382 and .0552). Large indigestible cell wall residues (45.78% to 70.83%), small amounts of potentially digestible cell wall (27.19% to 48.19%) and low to moderate rate constants (.0185 to .0492) were observed for waxed corrugated papers and newsprint. The large rate constants were similar to those determined by forages (Smith et al, 1972; Mertens, 1973; Robles, 1977).

Lastly, lag time was twelve hours for the four corrugated papers which is notably greater than the three hour lag observed for forages (Mertens, 1973; Robles, 1977). The other papers had shorter lags similar to the lag of forages (three to six hours).

CHAPTER III

MATERIALS AND METHODS

1. Design of Sustained Release Delivery System

The basic design of the Sustained Release Delivery System (SRDS) was a cylinder shaped bolus, measuring 2.54 cm in diameter, with alternating paper membrane and paper spacers. This configuration provided a multicompartmental sequentially alligned arrangement. The thickness of the spacers used to divide the paper membranes depended on the quantity of material to be released in a dose. Using five spacers to form a compartment within the bolus allowed ample capacity for holding 300 mg of most pharmaceuticals of interest. The end of the SRDS was exposed to allow degradation of paper membranes by rumen microbes. A steel ball measuring 2.54 cm in diameter was attached to the opposite end of the SRDS so that the SRDS would be lodged in the reticulo-rumen and not be regurgitated. The outside of the SRDS was coated with a flexane-urethane compound[1] which functioned to maintain the shape of the SRDS and to prevent penetration of rumen fluids except from the exposed end. The SRDS was modified to some extent in each trial. Elaboration of trials will include detailed modifications of the SRDS.

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[1]Devcon Corporation, Danvers, Massachusettes

2. General Description of Trials Conducted

A total of ten *in vivo* trials were employed to study the SRDS. Eight of these trials were conducted using ruminally fistulated animals. SRDS's were placed in the rumens via cannulas. In the trials involving the fistulated animals, the SRDS's were periodically removed and observations were recorded, particularly the rate of release. Paper membranes were sequentially numbered to indicate the rate of degradation of the SRDS in the reticulo-rumen. Upon removal of the SRDS from the rumen, the rate of release was determined by the number of paper membranes degraded per day. The purpose of these experiments was to determine the effect of paper membrane type, type of covering on exposed end, kind of substrate release, individual animal variation and diet upon the rate of release of the SRDS. The other two trials involved work with nonfistulated cows. In these two trials, pharmaceuticals (cromium oxide and Rumensin ^{R} were incorporated into the SRDS's in order to determine the releasing capabilities of the delivery system.

Trials 1, 2, 5 and 6 were conducted using one ruminally fistulated steer. Trials 7, 8, 9 and 10 were conducted using six ruminally fistulated Jersy females, and Trials 3 and 4 were conducted using nonfistulated cows. All trials were carried out at The University of Tennessee's Knoxville Experiment Station with

the exception of Trial 4 which was performed at the Plateau Experiment Station in Crossville. These trials began in 1980 and were concluded in 1983.

3. Detailed Description of Each Trial

Trail 1 (August, 1980)

There are numerous arrays of membrane types available commercially in the paper industry. The purpose of this trial was to determine if variation of rate of exposure would exist with different paper weights (kilogram basis) within one paper type. The kilogram (Kg) basis of kraft paper is defined as the weight of 500 sheets of 8-1/2 x 11 paper. The paper used to test one type was brown kraft and the different weights tested were 34.1, 31.8, 27.2, 22.7, 18.2 and 13.6 Kg basis, (Table 2).

Table 2. Influence of Differnt Weights Within One Paper Type on Rate of Exposure of SRDS.

	Weight of Paper (Kg Basis)					
	34.1	31.8	27.2	22.7	18.2	13.6
Number Of SRDS's	16	3	2	3	2	3

Twenty-nine SRDS's were fabricated in accordance with the SRDS design description and subsequently placed in the rumen via the cannula. Periodic observations to determine the rate of release of SRDS's in the reticulo-rumen were made at intervals ranging from six to 30 days.

Trial 2 (September, 1980)

The purpose of this trial was to determine the variation of rate of exposure of different paper weights (Kg basis) among different paper types. Twenty-one SRDS's were constructed in the same fashion as in Trial 1 except different paper membrane types were tested. Paper types tested were waterproof (14.5 Kg basis; Kg basis for waterproof paper is defined as the weight of 500 sheets of 17 x 22 paper), brown kraft (18.2 and 34.1 Kg, respectively) and parafin, (Table 3).

Table 3. The Effect of Different Paper Weights Among Different Paper Types on Rate of Exposure of SRDS

	Paper Type: Weight of Paper (Kg Basis)			
	Waterproof 100% Rag	Brown Kraft 18.2 Kg Basis	Brown Kraft 34.1 Kg Basis	Parafin
Number Of SRDS's	6	8	6	2

The fistulated steer used in Trial 1 was also used in this experiment. The steer was maintained in fescue pasture during the month of November. SRDS's were observed for the rate of release at intervals ranging from four to 15 days in the reticulo-rumen.

Trial 3 (August--October, 1981)

This trial was conducted to study the effect of diet and animal variation on releasing capabilities of the SRDS. Twenty-four SRDS's were designed the same as they were in Trials 1 and 2 with the following modifications: 34.1 Kg brown kraft paper was used for paper membranes and cromic oxide was measured into each compartment (90 compartments) of the SRDS at a rate of 250 mg per compartment. In the previous trials, "packing" of rumenal digesta in the SRDS was observed. Packing or clogging of the delivery system by digesta was apparently responsible, in part, for degradation rates of the membranes. Hence, in an attempt to alleviate this problem, the exposed end was covered with a net material.

Twenty-four nonfistulated mature Angus, Herferd and Angus Herferd cows were used for this experiment. These cows were fed the following diets in individual pens: Twelve cows were fed grass silage ad libidum, six were fed 80% of the ad libidum amount, and six were fed grass silage ad libidum plus two pounds concentrate (six part corn, one part cotton seed meal/animal/day).

Each cow was orally administered, by way of a speculum, one SRDS which had been lubricated with mineral oil. For a 30 day period, fecal samples were collected on alternate days and analyzed for cromium. This trial was designed to last for 90 days, however, due to complications described in the Results and Discussion section of this paper, the trial had to be terminated after 30 days.

Trial 4 (April--July, 1980)

The objective of this experiment was to study the release of Rumensin® from an SRDS. Additionally, daily grain, hay consumption and feed efficiency was recorded for each animal to substantiate the theory that Rumensin® improves animal performance.

Forty-eight SRDS's were assembled using brown kraft paper (18.2 Kg basis) for paper membranes. Rumensin® 60 was incorporated into SRDS's at a rate of 150 mg per compartment, 90 compartments per SRDS. Twenty-four heifers averaging 284 Kg were individually fed 2.7 Kg of corn and .77 Kg supplement/heifer/day as well as orchard grass, white clover hay ad libidum for 86 days. Heifers were randomly allotted to treatments (Table 4).
The supplement fed to the heifers in the three treatments was identical except for the presence (or absence) of Rumensin® . All heifers receiving Rumensin® either via SRDS (orally administered

Table 4. Experimental Design of Release of Rumensin® From SRDS

	Treatment		
	Supplement With No Rumensin	Supplement With Rumensin	SRDS Plus Supplement With No Rumensin
Number of Cows/Treatment	8	8	8

with a speculum and mineral oil) or supplements were scheduled to receive 300 mg/heifer/day. Each heifer receiving SRDS containing Rumensin® received two SRDS's in order to obtain the desired release of 300 mg/heifer/day.

Trial 5 (November, 1980)

This trial was employed to continue the study of rate of release of the SRDS. The packing of ingesta inside the exposed end of the SRDS was still a problem. In this trial, the SRDS was modified slightly by covering the exposed end with nylon material which had a smaller, denser mesh than the net material used in

Trial 3. In theory, the smaller, denser mesh would function to prevent large digesta particles from entering the exposed end of the SRDS.

The ruminally fistulated steer used in Trials 1 and 2 was managed in the same manner as in Trials 1 and 2. A total of 28 SRDS's made with 18.2 Kg basis brown kraft paper were constructed to last 40 days (40 compartments/SRDS). Thirteen SRDS's were made with nylon covering on the exposed end and compared to 15 SRDS's with no covering. Observations pertaining to the rate of exposure and packing in the reticulo-rumen were recorded for a period of six to 41 days. This was accomplished by manually removing the SRDS's and recording the number of paper membranes that had been degraded.

Trial 6 (December--February, 1981)

This trial was similar to the first three trials with the following modifications: Two types of covering, nylon and net, were compared to no covering on the exposed end of the SRDS. Delivery systems composed of 34.1 Kg basis brown kraft paper membranes were made to degrade in 20, 40 and 60 days (20, 40 and 60 compartments/SRDS). Observing SRDS's at 60 days in the reticulo-rumen, as compared to a maximum of 41 days in the reticulo-rumen from previous trials, allowed determination of change in rate of exposure over a longer period of time. Six, 20-day (20 compartments/SRDS); six, 40-day (40 compartments/SRDS); and six, 60-day (60 compartments/SRDS) were constructed, (Table 5).

Table 5. The Effect of Different Covering Types Over the Exposed End of SRDS on the Rate of Exposure of Paper Membranes Contained in SRDS's

	Number of Compartments/SRDS		
Type of Covering	20	40	60
No Covering	2	2	2
Coarse Net	2	2	2
Dense Net	2	2	2

The fistulated steer was maintained on fescue hay fed ad libidum throughout the trial. SRDS's remained in the reticulo-rumen for a minimum of 24 days and a maximum of 67 days. SRDS's were manually removed via the cannula and observed for rate of exposure and packing of ingesta in the exposed end.

Trial 7 (August--September, 1982)

Six fistulated Jersey females, weighing approximately 273 Kg, were used in an experiment designed to test the rate of exposure of waterproof paper. The effect of diet and variaiton among and within the animals, on the rate of release of SRDS's were studied. Surgery to cannulate the females was conducted 27 days prior to the beginning of the trial. At this time, three of the Jerseys were

found to be pregnant and were aborted. During surgery, all Jerseys were also ovarectomize. The females were allotted randomly to two pasture treatments. Treatments were 60% fescue, 40% legume (red clover, Korean and Kobe lespedesa) and tall fescue. The SRDS's were designed in the same manner as in the previous trials. Thirty SRDS's were composed of 90 compartments made with waterproof paper with the exposed end covered with net material. In earlier trials, SRDS's which had been made with brown kraft paper were retrieved and cut open. It was noted that rumen fluids were prematurely saturating papers throughout the length of the SRDS. For this reason, waterproof paper was used in constructing the SRDS's in this trial to alleviate premature saturation of subsequent exposed membranes by rumen fluids. Waterproof paper had been previously used in Trial 2 in the construction of SRDS's. However, at this time, waterproof paper was used only as a variety of paper types studied.

Each female received five identical SRDS's via the cannula. On day 26 of the trial, SRDS's were manually retrieved via the cannulas and observed for rate of exposure, packing of ingesta in the exposed end, and premature saturation by rumen fluids of unexposed paper membranes. At this time, several SRDS's were unattainable; some SRDS's had been passed or regurgitated. Thus, the trial was terminated.

Trial 8 (October--December, 1983)

This trial was similar to Trial 7 except that the SRDS's remained in the reticulo-rumen for a longer period of time (71 days versus 26 days in Trial 7). The ruminally fistulated Jersey females used in Trial 7 were also used in this experiment to study the effect of diet and animal variation on the rate of release of SRDS's composed of waterproof paper. Treatments (60% fescue, 40% legume and tall fescue) were also the same as the previous trial. Each female received five identical SRDS's (90 compartments composed of waterproof paper with the exposed end covered with net material) via the cannula. Subsequently, SRDS's were manually collected through the cannula and observed for rate of exposure, packing of ingesta in the exposed end, and were cut open to determine if premature saturation by rumen fluids of unexposed paper membranes existed.

Trial 9 (January--March, 1983)

The objective of this experiment was to evaluate consistency of release of Ciba-Geigy A-72662 (an experimental compound being investigated for insecticide properties) from a SRDS. Six ruminally fistulated Jersy females from the previous trial were utilized for this trial. Ninety-day (90 compartments) SRDS's were fabricated using waterproof paper membranes with net material covering the exposed end. Each compartment contained 150 mg CGA-72662. The

experimental design consisted of three treatments (two females per treatment). The treatments were: Corn silage fed ad libidum plus one pound cotton seed meal (CSM)/head/day; the second treatment was the same as the first except the CSM was mixed with the insecticide at a rate of 150 mg CGA-72662/100 pounds CSM. In addition to silage plus one pound CSM/head/day, females in treatment three received one SRDS each containing CGA-72662. In order to test the rate of degradation of SRDS's for all females, each female received two SRDS's. These SRDS's were composed of waterproof paper membranes and coarse net covering over the exposed end. CGA-72662 was to be administered to the animals at a rate of 150 mg/273 Kg animal/head/day. The Jerseys were weighed at the beginning of the trial and assigned to treatment according to body weight. Fecal samples were collected from each animal on alternate days during the first and last two weeks of the trial. Fecal samples were also collected once a week per animal during other weeks. The trial was scheduled to last 90 days, however, the trial was terminated on day 41 because the SRDS's became packed with digesta and were no longer releasing the insecticide. At this time, SRDS's were removed and rate of degradation was recorded.

Trial 10 (March--April, 1983)

Throughout the trials previously described, the problem of packing of digesta in the exposed end of the SRDS persisted to a variable degree. This trial involved the evaluation of a modification of the

design to overcome this problem. The modified SRDS was similar to the original model with the following alterations: Both ends of the SRDS were exposed to microbial degradation and the steel ball was attached to the center of the SRDS by a nylon cord measuring approximately 7.5 cm in length. This allowed the SRDS to float approximately 7.5 cm from the floor of the rumen in addition to allowing the SRDS to shift from end to end in synchronization with natural rumen motility. Two SRDS's using waterproof paper were constructed to compile 40 compartments. These modified SRDS's were placed in the rumens of females from Trial 8 who originally had SRDS's containing CGA-72662 (treatment three). The other two treatments were exactly the same as in Trial 8, however, no SRDS's containing waterproof paper were placed in the females to compare the rate of degradation. Fecal samples were collected once a week for four weeks. Delivery systems remained in the rumen for 31 days at which time they were removed and examined for rate of exposure and packing of ingesta.

4. Statistical Analysis

Data from this research was statistically analyzed by trial. For Trials 1, 2, 5 and 6, analysis of variance were performed on the dependent variables: Day, cover type, paper type and basis. The model, or variations of the model, EXPO=day, cover type, paper type, basis, day x cover type, day x paper type, day x basis,

cover type x paper type, cover type x basis, paper type x basis were used to obtain values for least square means for each variable.

For Trial 4, the model Y=weight, April 21 + treatment was employed. If differences were determined by F test, least square means were separated using Duncans New Multiple Range test (Snedecor and Chochran, 1967).

For Trials 7 and 8 a split plot statistical design with treatment in the main plot and animal in the subplot was used.

Analysis of variance was performed on dependent variables: Day, treatment and animal number. The model, exposed/day=day, treatment, day x treatment animal number (treatment), animal number (treatment) x day was used to obtain values for standard errors and least square means.

CHAPTER IV

RESULTS AND DISCUSSION

1. Results Discussed by Trial

Trials 1, 2, 5, and 6

These trials, using one ruminally fistulated steer, were conducted to determine if the following variables had an effect on the rate of exposure of SRDS(s), paper weight (Kg basis) within one paper type, paper weights (Kg basis) among different paper types, and different covering types (coarse vs dense vs none) used to cover the exposed end of SRDS (s). Results from these trials indicated paper weight (Kg basis) basis had a significant effect ($P<.001$) on rate of exposure of SRDS (Tables 6 and 7). There was a slight trend for thinner paper (13.6 Kg basis) to degrade at a faster rate than thicker paper (31.8 Kg basis) (.60 paper membranes exposed per day vs .47 paper exposed per day) membranes with the exception of 34.1 Kg basis paper which had .72 paper membranes exposed per day.

Moreover, there is a considerable amount of variation in rate of exposure between different paper types and weights (Kg basis). This variation may be partially due to packing of ingesta in the exposed end of the SRDS. It is possible that some SRDS(s) may have been situated in the reticulo-rumen in such a way that packing of ingesta in the forming cavity was not as pronounced as in other SRDS(s).

Covering type also had a significant effect on rate of exposure of SRDS(s) ($P<.01$) (Tables 6 and 7). The interaction of covering

Table 6. Analysis of Variance of Paper Weights (basis) and Covering Type (C type) effects on Exposure Rate of Slow Release Delivery System (SRDS)

Source	df	MS	PR>F
Day	1	.3479	.0586
Ctype	2	.6414	.0020
Basis	5	.4307	.0012
Day x Ctype	2	.7564	.0007
Day x basis	5	.2617	.0237
Ctype x basis	1	1.7983	.0001
Residual	71	.0942	
R^2 = .4771			

Day = no. of days SRDS remained in reticulorumen; C type 1 = dense net; Ctype 2 - no covering; Ctype 3 - coarse net; basis = weight of paper (kg basis).

Table 7. Influence of Paper Weight and Covering Type on Exposure Rate of SRDS(s)[a]

Covering type (Ctype)	Paper weight (Kg basis)					
	13.6	18.2	22.7	27.2	31.8	34.1
1		.94				
2	.60	.59	.23	.39	.47	.72
3						.64

[a]Least square means from the model EXPD=Day, Ctype, Basis, Day x Ctype, Day x Basis, Ctype x Basis.

Ctype 1 = dense net covering; Ctype 2 = no covering; Ctype 3 = coarse net covering.

types and basis was highly significant (P<.0001). Specifically, SRDS's constructed with 18.2 Kg basis paper degraded more rapidly than 34.1 Kg basis paper when dense net material was used to cover the exposed end. Reasons for these outcomes are not clear; however, it should be noted that securing a cover over the exposed end of SRDS(s) did not alleviate the problem of packing of ingesta.

Day also had a significant effect on the rate of exposure of SRDS (Table 6). There was a curvilinear response to the rate of exposure of paper membranes with respect to the number of days SRDS(s) remained in the reticulo-rumen (Table 8). Thus, the rate of paper membranes exposed per day decreased as the time SRDS(s) remained in the animal increased. This effect may be attributed to packing of ingesta in the exposed ends of SRDS(s) which presumably caused the depression in rate of exposure paper membranes contained within the delivery systems.

Table 8. Analysis of Variance of the Effect of Day on Rate of Exposure of SRDS

Source	DF	MS	PR>F
Day	1	1.5359	0.0001
Day x Day	1	1.0382	0.0002
Residual	36	0.0602	

R^2 = .4716

Trial 3

The trial was employed to observe the release of cromium oxide from a SRDS. No cromium oxide was detected in the fecal samples which were collected from all cows every other day for the first thirty days of this trial. Additionally, two cows regurgitated or passed their SRDS(s). Examination of the SRDS(s) revealed packing of ingesta in the exposed end of both delivery systems. Apparently, packing of ingesta in the exposed end of the SRDS(s) prevented the degredation of paper membranes and the release of chromium oxide. Results from cutting open the recovered SRDS(s) revealed premature saturation of unexposed paper membranes (brown kraft 34.1 Kg basis paper) by rumen fluids. Hence, this trial was terminated and data were not statistically analyzed.

Trial 4

The purpose of this trial was to determine the rate of release of Rumensin® from a SRDS and the effect of Rumensin® on performance. Each animal's pen was searched daily for SRDS that had been either regurgitated or passed. On May 29, remnants of two SRDS(s) were found. Evidently the flexane-urethane compound used in this trial eroded resulting in a breaking-up of the SRDS. In later studies, this problem was eliminated by using a harder, less flexible flexane-urethane compound. No other SRDS were found. One of the two heifers that lost her SRDS was rebolused with 2 SRDS(s) on May 29.

Experimental results are presented in Table 9. Due to uncertainty concerning the two heifers that lost SRDS, data both including them

Table 9. Performance of Animals Receiving Rumensin® in Feed or Via Slow Release Delivery

Item	Treatment				
	Bolus + supplement with no Rumensin®	Supplement with 30 mg Rumensin®/day	Supplement with no Rumensin®	Level of significance of F	RSD[c]
All heifers					
Weight, April 21	286[a]	283[a]	282[a]	.96	67.3
Daily gain for 86 days (Kg/heifer/day)	.814[a]	.864[a]	.755[a]	.32	.32
Daily gain for last 33 days (Kg/heifer/day)	.645[a]	.586[a]	.441[a]	.23	.535
Hay consumption for 86 days (Kg hay)	374	270	359	.09	211.5
Feed efficiency for 86 days (lb hay/lb gain)	4.88[a]	3.70[b]	5.59[ab]	.01	1.213
Number	8	8	8		
Only heifers that retained boluses					
Weight, April 21	272[a]	283[b]	282[b]	.66	65.9
Daily gain for 86 days (Kg/heifer/day)	.87[a]	.86[a]	.75[a]	.18	.290
Daily gain for last 33 days (Kg/heifer/day)	.71[a]	.59[a,b]	.44[b]	.05	.525
Hay consumption for 86 days (Kg hay)	380[a]	270[a]	359[a]	.08	217.4
Feed efficiency for 86 days (lb hay/lb gain)	4.88[a]	3.70[b]	5.59[ab]	.02	1.214
Number	6	8	8		

Table 9 (Continued)

[a,b]Means on the sam row with different superscripts are different (P .05) according to Duncan's New Multiple Range Test.

[c]Residual standard deviation from model: Y = treatment when all heifers included and Y = weight, April 21 + treatment when only heifers that retained boluses included.

[d]Least square means from model: Y = weight, April 21 + treatment.

and excluding them were reported. In general, gain data were measured more precisely than feed efficiency data, and therefore provide a more exacting test of the efficacy of the SRDS. For the six heifers that apparently retained the SRDS for the duration of the trial, daily gain was similar ($P>.05$), if not better, than heifers fed Rumensin® in supplement. This finding was apparent throughout the entire trial. The performance during the last 33 days of the trial indicated the SRDS were still releasing Rumensin® during that time. Furthermore, the increased performance of the six heifers that retained the SRDS for the duration of the trial, as compared to the two that did not, indicates the efficacy of the SRDS. Heifers that received Rumensin® (either by SRDS or by supplement) had increase in gain within the range experienced by other workers. Muller et al. (1980) have shown that Rumensin® does not improve performance unless given to animals in a controlled manner daily or at least on alternate days. Therefore, the SRDS must have been releasing rumensin very close to the desired dosage rate.

The feed efficiency data shown in Table 9 indicates the same data trends as seen in gain data; Rumensin® treated heifers (either via SRDS or via supplement) were more efficient than heifers who received no Rumensin® (4.88, 3.70, and 5.59 Kg/heifer/day, respectively). Feed efficiency is calculated as Kg hay/Kg gain because other components of the diet were fed at a constant rate.

Trials 7 and 8

Six fistulated Jersey females were used in these experiments designed to test the effect of diet and variation, among and within animals, on the rate of exposure of SRDS. Two animals from these trials regurgitated or passed their SRDS(s). It is possible the weights designed to retain the SRDS in the reticulo-rumen were not tightly secured to the ends of these SRDS(s) resulting in passage or regurgitation of the body of the SRDS from these animals. These animals were deleted from the data.

Results from this study revealed some variation existed between SRDS on rate of exposure (Table 10). In addition, as the number of days SRD(s) remained in the reticulo-rumen increased, the number of paper membranes exposed per day decreased ($P<.05$). Analysis of these trials indicated there was a curvilinear response to rate of exposure of paper membranes over time (Table 11). These results coincide with the outcome of trials 1, 2, 5, and 6 in the rate of exposure of SRDS(s) decreased over time. Again, this effect may be ascribed to packing of ingesta in the exposed end of the SRDS(s). A method to alleviate packing of ingesta in the exposed end may remove this curvilinear response contributed by day.

Diet (fescue legume vs fescue) had no effect on the rate of exposure of SRDS ($P>.1$) (Table 9). This finding is not surprising for the number and kinds of bacteria found in the reticulo-rumen of the bovine species do not differ on rations other than concentrate (Bryant and Burkey, 1957). It is possible, however, that the

Table 10. Least Square Means for Day, Animal and Treatment

Day	Animal No.	Treatment	Exposed/day LS means	Std err LS means
26	508	1	.63	.076
26	821	1	.57	.054
57	508	1	.51	.062
57	821	1	.46	.062
71	521	1	.56	.107
71	821	1	.62	.107
26	798	2	.63	.054
26	814	2	.54	.048
26	841	2	.67	.076
50	814	2	.38	.048
57	798	2	.48	.076
57	841	2	.61	.076

Treatment 1 = fescue-legume (red clover, white clover, Korean and Kobe lespedesa), Treatment 2 = tall fescue.

Table 11. Analysis of Variance Relating Variation in Time to Reticulo-rumen Pasture Type and Animal to Rate of Exposure

Source	df	MS	PR>F
Day	3	.0329	0.0583
TMT	1	.0029	0.6202
Day x TMT	1	.0018	0.6972
ANNO (TMT)	4	.0137	0.3402
Day x ANNO (TMT)	3	.0048	0.7426
Residual	24	.0115	
R^2 = .4812			

Treatment 1 = fescue legume (red clover, white clover, Korean and Kobe lespedesa); treatment 2 = tall fescue.

rate of exposure of SRDS(s) may differ if some animals were fed concentrate diets and compared to animals fed forage diets. This area of study needs further investigation.

There was no variation among and within animals on the rate of exposure of SRD(s) ($P>.1$) (Table 10).

Trial 9

The objective of this experiment was to evaluate the consistency of release of a fly insecticide (Ciba-Giegy A-72662) from a SRDS. The problem of packing of ingesta in the exposed end of insecticide containing SRDS(s) became apparent after ten days of this trial. The trial was continued for thirty-two more days, at which time, SRDS(s) were removed from all animals. Observations of SRDS(s) revealed packing of ingesta in the exposed end had persisted. Therefore, this trial was terminated and rescheduled to be conducted at a later date fabricating SRD's designed to overcome the problem described above.

Trial 10

A modified SRDS, designed to alleviate the problem of packing of ingesta in the exposed end. was tested in this experiment. Both ends of the SRDS were exposed to microbial degredation and the steel ball was attached to the center of the SRDS by a nylon chord measuring 7.5 cm in length. Data collected from this trial used to test a design modification of SRDS(s) was not statistically analyzed. However, SRDS(s) were observed for rate of exposure and packing of

ingesta in the exposed end. No digesta was found in the SRDS's. Paper membranes were evenly degrated from each opened end of the SRDS's, and were exposed at a rate of 1.1 paper membranes exposed per day. Fecal samples collected from these animals have not been analyzed for ovicidal properties to this date. Completion of fecal analysis would give a relatively good estimate of the daily effective release of the insecticidal compound from the modified SRDS.

Conclusion

Overall, a considerable amount of variation existed in the rate of exposure of slow release delivery systems. Factors such as the type of paper used and the thickness of the paper may be, in part, responsible for differences seen in rate of exposure between SRDS's. More importantly, the packing of ingesta in the exposed ends of SRDS's was probably the main reason for failure of SRDS's to release at a consistant rate over time: rate of exposure of SRDS's decreased over time. Day effect was significant throughout all trials.

Interestingly, the SRDS(s) which were successfully modified to alleviate the problem of packing of ingesta in the exposed end, showed considerable promise for achieving daily release of the SRDS. If the problem of packing of ingesta has indeed been solved, then perhaps other variables affecting the rate of exposure could be more clearly understood in future studies.

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APPENDIX

Table A1. Data Collected From Trials 1, 2, 5, and 6

OBS	ID	PTYPE	CTYPE	EXP	DAY	BASIS	TRIAL	EXPD
1	1	2	2	12	29	75	1	0.41379
2	2	2	2	4	6	75	1	0.66667
3	3	2	2	9	8	75	1	1.12500
4	4	2	2	10	16	75	1	0.62500
5	5	2	2	14	30	75	1	0.46667
6	6	2	2	12	35	75	1	0.34286
7	7	2	2	10	8	75	1	1.25000
8	8	2	2	10	16	75	1	0.62500
9	9	2	2	10	30	75	1	0.33333
10	10	2	2	8	8	75	1	1.00000
11	11	2	2	6	10	75	1	0.60000
12	12	2	2	6	19	75	1	0.31579
13	13	2	2	11	10	75	1	1.10000
14	14	2	2	3	19	75	1	0.15778
15	15	2	2	6	10	75	1	0.60000
16	16	2	2	5	19	75	1	0.26316
17	17	2	2	6	10	70	1	0.60000
18	18	2	2	9	19	70	1	0.47368
19	19	2	2	6	10	70	1	0.60000
20	20	2	2	10	10	60	1	1.00000
21	21	2	2	8	19	60	1	0.42105
22	22	2	2	9	10	50	1	0.90000
23	23	2	2	10	10	50	1	1.00000
24	24	2	2	5	19	50	1	0.26316
25	25	2	2	8	10	40	1	0.80000
26	26	2	2	6	19	40	1	0.31579
27	27	2	2	12	10	30	1	1.20000

Table A1 (Continued)

OBS	ID	PTYPE	CTYPE	EXP	DAY	BASIS	TRIAL	EXPD
28	28	2	2	11	10	30	1	1.10000
29	29	2	2	12	19	30	1	0.63158
30	30	1	2	13	4	.	2	3.25000
31	31	1	2	13	4	.	2	3.25000
32	32	1	2	12	15	.	2	0.80000
33	33	1	2	11	15	.	2	0.73333
34	34	1	2	12	15	.	2	0.80000
35	35	1	2	11	15	.	2	0.73333
36	36	2	2	9	4	40	2	2.25000
37	37	2	2	8	4	40	2	2.00000
38	38	2	2	9	9	40	2	1.00000
39	39	2	2	8	9	40	2	0.88889
40	40	2	2	7	9	40	2	0.77778
41	41	2	2	9	9	40	2	1.00000
42	42	2	2	8	9	40	2	0.88889
43	43	2	2	10	9	40	2	1.11111
44	44	2	2	8	9	75	2	0.88889
45	45	2	2	9	9	75	2	1.00000
46	46	2	2	10	9	75	2	1.11111
47	47	2	2	10	9	75	2	1.11111
48	48	2	2	14	15	75	2	0.93333
49	49	2	2	16	15	75	2	1.06667
50	50	3	2	2	4	75	2	0.50000
51	51	3	2	1	4	75	2	0.25000
52	52	2	1	8	8	40	3	1.00000
53	53	2	1	6	9	40	3	0.66667
54	54	2	1	12	10	40	3	1.20000
55	55	2	1	11	10	40	3	1.10000
56	56	2	1	8	10	40	3	0.80000

Table A2 (continued)

OBS	ID	PTYPE	CTYPE	EXP	DAY	BASIS	TRIAL	EXPD
57	57	2	1	10	10	40	3	1.00000
58	58	2	1	9	10	40	3	0.90000
59	59	2	1	9	10	40	3	0.90000
60	60	2	1	11	10	40	3	1.10000
61	61	2	1	13	12	40	3	1.08333
62	62	2	1	13	15	40	3	0.86667
63	63	2	1	44	41	40	3	1.07317
64	64	2	1	37	41	40	3	0.90244
65	65	2	2	4	6	40	3	0.66667
66	66	2	2	9	9	40	3	1.00000
67	67	2	2	13	9	40	3	1.44444
68	68	2	2	10	9	40	3	1.11111
69	69	2	2	10	16	40	3	0.62500
70	70	2	2	13	18	40	3	0.72222
71	71	2	2	3	9	40	3	0.33333
72	72	2	2	5	19	40	3	0.26316
73	73	2	2	9	19	40	3	0.47368
74	74	2	2	8	19	40	3	0.42105
75	75	2	2	8	19	40	3	0.42105
76	76	2	2	5	19	40	3	0.26316
77	77	2	2	6	19	40	3	0.31579
78	78	2	2	12	19	40	3	0.63158
79	79	2	2	14	41	40	3	0.34146
80	80	2	2	26	45	75	4	0.57778
81	81	2	2	52	67	75	4	0.77612
82	82	2	3	35	45	75	4	9.77778

Table A2 (Continued)

OBS	ID	PTYPE	CTYPE	EXP	DAY	BASIS	TRIAL	EXPD
83	83	2	3	35	45	75	4	0.77778
84	84	2	1	46	67	75	4	0.68657
85	85	2	1	11	35	75	4	0.31429
86	86	2	2	24	24	75	4	1.00000
87	87	2	2	4	45	75	4	0.08889
88	88	2	3	18	45	75	4	0.40000
89	89	2	3	18	45	75	4	0.40000
90	90	2	1	6	45	75	4	0.13333
91	91	2	1	5	45	75	4	0.11111
92	92	2	2	20	28	75	4	0.71429
93	93	2	3	20	28	75	4	0.71429
94	94	2	3	15	28	75	4	0.53571
95	95	2	1	9	28	75	4	0.32143
96	96	2	1	8	28	75	4	0.28571

Table A3. Data Collected From Trials 7 and 8

OBS	ID	PTYPE	CTYPE	EXP	DAY	TRIAL	COWNO	TMT	EXPD
1	97	1	3	13	26	5	841	1	0.500000
2	98	1	3	22	26	5	841	1	0.846154
3	99	1	3	18	26	5	798	1	0.692308
4	100	1	3	16	26	5	798	1	0.615385
5	101	1	3	15	26	5	798	1	0.576923
6	102	1	3	16	26	5	798	1	0.615385
7	103	1	3	14	26	5	814	1	0.538462
8	104	1	3	14	26	5	814	1	0.538462
9	105	1	3	13	26	5	814	1	0.500000
10	106	1	3	17	26	5	814	1	0.653846
11	107	1	3	12	26	5	814	1	0.461538
12	108	1	3	16	26	5	821	2	0.615385
13	109	1	3	16	26	5	821	2	0.615385
14	110	1	3	16	26	5	821	2	0.615385
15	111	1	3	16	26	5	821	2	0.615385
16	112	1	3	11	26	5	321	2	0.423077
17	113	1	3	15	26	5	321	2	0.576923
18	114	1	3	18	26	5	321	2	0.692308
19	115	1	3	30	57	6	841	1	0.526316
20	116	1	3	40	57	6	841	1	0.701754
21	117	1	3	22	57	6	798	1	0.385965
22	118	1	3	33	57	6	798	1	0.578947
23	119	1	3	16	50	6	814	1	0.320000
24	120	1	3	17	50	6	841	1	0.340000
25	121	1	3	13	50	6	798	1	0.260000
26	122	1	3	21	50	6	798	1	0.420000
27	123	1	3	29	50	6	814	1	0.580000

Table A3 (Continued)

OBS	ID	PTYPE	CTYPE	EXP	DAY	TRIAL	COWNO	TMT	EXPD
28	124	1	3	23	57	6	821	2	0.403509
29	125	1	3	35	57	6	821	2	0.614035
30	126	1	3	44	71	6	821	2	0.619718
31	127	1	3	39	57	6	821	2	0.684211
32	128	1	3	29	57	6	508	2	0.508772
33	129	1	3	29	57	6	508	2	0.508772
34	130	1	3	30	57	6	508	2	0.526316
35	131	1	3	20	57	6	521	2	0.350877
36	132	1	3	29	57	6	521	2	0.508772
37	133	1	3	29	57	6	521	2	0.508772
38	134	1	3	40	71	6	521	2	0.563380

VITA

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