TECHNIQUES FOR DETERMINING THE RATE OF METABOLISM OF C^{14} LABELED ALFALFA IN THE BOVINE RUMEN

by

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INTRODUCTION

The fate of dietary carbohydrates in the reticulo-rumen (herafter referred to as rumen) of cattle is considerably different from that in the stomach of a monogastric animal. Instead of a simple hydrolysis which leads to a rapid absorption of glucose, much of the carbohydrate material is broken down to volatile fatty acids (VFA) in the rumen. A substantial portion of the ruminant's energy requirement is met by the absorption of VFA produced in the rumen. The breakdown of sugars and starch to VFA in the rumen might be considered a somewhat inefficient process when compared with digestion further on down the digestive tract; however, the breakdown of cellulose to VFA in the rumen is essential for maximum utilization because only small amounts of cellulose can be degraded in the lower digestive tract.

Cellulose is present in all ruminant rations and in many cases it is the primary energy source for the animal. A knowledge of when and how cellulose is degraded in the rumen could provide improved feeding methods which would increase the utilization of cellulose. This could be of great economic importance because enormous quantities of cellulose are utilized by ruminants.

Various in vitro artificial rumen techniques have been employed to determine the rate and extent of cellulose degradation by rumen microorganisms. Attempts have been made to simulate rumen conditions as closely as possible; however, the complexity of the ruminant digestive system makes this a difficult task. There is much disagreement in the literature as to when cellulose digestion occurs. This indicates that in vitro techniques may not duplicate rumen conditions well enough to determine the rate of cellulose digestion.

In vivo techniques also have been used to study cellulose digestion.

These methods are not subject to the same limitations as those of in vitro methods, and it is possible to determine the extent of cellulose digestion. However, it is difficult to study rate of digestion because the cellulose in a given feeding loses its identity when it enters the rumen. Cellulose digestion is believed to be a slow process, so cellulose from several previous feedings may be present in the rumen at any given time. Consequently, a method of identifying the cellulose from a given feeding is essential to study the rate of cellulose digestion in vivo.

To overcome the limitations of in vitro and in vivo techniques, a new approach using a common roughage such as alfalfa hay labeled with C^{14} appears to have merit. A small amount of labeled alfalfa hay placed in the rumen should be degraded along with any other unlabeled hay fed. The appearance of labeled VFA in the rumen fluid can be detected by radiological methods and thus indicate when the labeled hay is degraded.

The most desirable method of determining the rate of cellulose digestion would be to label only the cellulose in alfalfa hay. At the present time selective labeling of the components of a roughage is impossible so it appears more desirable to use a uniformly labeled roughage than to use an extracted cellulose. Extracted cellulose is not a normal feedstuff and is more accessible to the action of rumen microorganisms than cellulose occurring in its natural form in the plant.

The criterion for degradation of labeled hay in these studies was the detection of C^{14} activity in the rumen VFA. It was recognized that there would be labeled VFA in the rumen that resulted from the degradation of the noncellulose portion of the labeled hay. Therefore the activity in the VFA would indicate the rate of breakdown of alfalfa hay, but not necessarily the rate of breakdown of cellulose; however, it was postulated that a general

idea on the rate of cellulose degradation could be obtained since it is generally accepted that cellulose is digested more slowly than other portions of hay. Prolonged activity in the VFA was expected to be a result of cellulose degradation.

REVIEW OF LITERATURE

Since the hay used in this study was uniformly labeled with C^{14} and there was no way of distinguishing between the VFA produced from its various components, a review of the literature pertaining to when the ruminal digestion of each component part of a roughage occurs would appear pertinent. Nearly all of the VFA produced in the rumen are a result of degradation of nitrogen free extract, crude protein, and crude fiber so each will be reviewed separately.

Digestion of Nitrogen Free Extract

According to Boggs et al. (1957) rumen microorganisms utilized the more soluble saccharides first and polysaccharides were broken down when the supply of readily available energy was reduced. Of the polysaccharides, starch was more readily available than cellulose. Barnett and Reid (1961) reported that the carbohydrates in the nitrogen free extract portion of a feed were rapidly metabolized by the rumen microflora, but the fiber was more slowly attacked.

A series of *in vivo* experiments by Phillipson and McAnally (1942) demonstrated the relationship between type of carbohydrate fed and rate of fermentation in the rumen of sheep. Rapidly fermented sugars such as glucose, fructose, and sucrose were readily broken down in the rumen while lactose, maltose, and galactose were much less efficiently utilized. Starch and

cellulose were attacked slowly. Hale et al. (1947) reported that the predominant phenomenon during the first 6 hr after feeding hay was the rapid disappearance of the more soluble nutrients, proteins, and carbohydrates from the rumen. Cellulose was only slightly digested during this period.

Digestion of Crude Protein

The breakdown of crude protein in the rumen results in the formation of ammonia and fatty acids. El-Shazly (1951) reported that the level of ammonia production was correlated with the increased concentration of branched chain fatty acids. It was concluded that the branched chain fatty acid fraction was formed due to microbial attack on protein and that protein was apparently broken down rapidly in the rumen. Using sheep fed a partially synthetic diet, Blackburn and Hobson (1960) demonstrated that the rate of breakdown of protein was related to solubility. Soluble proteins were rapidly degraded in the rumen and except for a short time immediately after feeding no measurable quantities were found in the rumen. Hale et al. (1947) showed that protein was rapidly degraded in the rumen during the first 6 hr after feeding. Protein digestion continued at a reduced rate for the next 6 hr. No protein was digested after 12 hr following feeding. Silver (1935) used a technique of sampling rumen contents before feeding alfalfa hay and sampling at 2 hr intervals after feeding. The sample taken before feeding contained a higher percentage of crude fiber and lower percentage of crude protein than the alfalfa hay fed. The sample taken 2 hr after feeding contained less crude fiber and more crude protein than the previous sample. Subsequent samples taken at 2 hr intervals showed a general rise in crude fiber and reduction in crude protein. It was concluded that the

more soluble nutrients pass out of the rumen faster than crude fiber.

Digestion of Crude Fiber

The major constituents of crude fiber are cellulose and lignin. Sullivan (1955) has demonstrated that lignin digestibility may sometimes be above 10%; however, Hale et al. (1940) (1947) and Balch (1957) have shown that lignin digestibility is small. Since it is generally agreed that lignin digestibility is small, the digestibility of crude fiber is primarily concerned with the breakdown of cellulose.

Digestion of Cellulose

There is disagreement in the literature concerning when cellulose is broken down in the rumen. Much of this difference of opinion is probably due to the diversity of in vitro techniques and extreme difficulty encountered when trying to simulate rumen conditions by laboratory methods. In addition the source of cellulose has an effect on experimental results. Extracted or purified cellulose is often used for in vitro fermentations. One would not necessarily expect that an extracted or purified cellulose would be digested at a similar rate as cellulose normally found in roughage.

Lag Phase. Many of the workers using in vitro techniques have reported a lag phase which must occur before cellulose digestion becomes apparent. The lag phase is defined as that period of time after inoculation of the in vitro fermentation in which no cellulose digestion is detectable. The lag phase usually varies from 0 to 12 hr. A recent review by Johnson (1963) indicates that the lag phase can be varied by changing a number of variables such as media, buffer system, particle size of cellulose substrate, and

inoculum preparation. Such a lag phase is naturally not apparent in the rumen because of close proximity to the steady state situation but still may exist on a single cellulose fiber newly ingested into the rumen.

A relatively short lag phase was demonstrated by Salsbury et al. (1958) because 4, 5, 8.3, 15.5, 6.9, and 10.3% of the cellulose in Solka Floc, cotton linters, alfalfa meal, wheat straw, and corncobs respectively were digested at the end of a 3 hr in vitro fermentation. Baumgardt et al. (1962) showed that about 5% of cellulose in orchard grass hay and 17% of cellulose in alfalfa hay were degraded at the end of a 6 hr in vitro fermentation.

A somewhat longer lag phase has been shown by other workers. Donefer et al. (1959) reported that the fermentation of nine different forage samples, representing three species of legume and two species of grass cut at various stages of maturity, demonstrated a lag phase of 2 to 6 hr in which no cellulose was degraded. Hamstra et al. (1958) using an in vitro technique demonstrated that there was very little cellulose digested in whole alfalfa during the first 7 hr of the fermentation. El-Shazly et al. (1961b) found that there was about a 6 hr lag phase for filter paper fermented in vitro. Dehority et al. (1961) reported that Solka Floc that had been wet-ball-milled for 6, 24, or 96 hr was not degraded during the first 6 hr of an in vitro fermentation.

A longer lag phase has been reported by some workers. El-Shazly et al. (1961a) found that there was no degradation of filter paper in vitro 12 hr following the start of the fermentation. Dehority et al. (1961) using Solka Floc cellulose (ground through a 100 mesh screen) demonstrated a lag phase of about 12 hr.

The results from in vivo experiments also suggest that the lag phase may be variable. Using a technique of complete removal of rumen contents Hale et al. (1947) reported that no cellulose was digested during the first 6 hr

following feeding. Yadava (1962) using an *in vivo* artificial rumen described by Fina *et al.* (1962) reported that no cellulose in whole alfalfa was degraded at the end of a 1 hr fermentation. Balch and Johnson (1950) demonstrated that there was very little digestion of cotton thread suspended in the rumen of a cow during the first 12 hr. A 12 hr lag phase was also reported by El-Shazly *et al.* (1961a) when filter paper in nylon bags was placed in the rumen of sheep.

Active Phase. The lag phase of cellulose digestion is usually followed by an active digestive phase. The length of time from the beginning of the active phase until cellulose digestion ceases is variable. Work by Donefer *et al.* (1959) indicated that the *in vitro* fermentation of both grass and legume forages exhibited a rapid rate of digestion from 6 to 24 hr. There was very little digestion of cellulose between 24 and 48 hr. Kamstra *et al.* (1958) noted a rapid breakdown of whole plant cellulose from 7 to 12 hr followed by slow breakdown from 12 to 24 hr with very little digestion from 24 to 30 hr. Baumgardt *et al.* (1962) using a high quality alfalfa hay *in vitro* reported a rapid breakdown of cellulose between 0 and 18 hr followed by gradually slower rate up to 42 hr at which time maximum digestion had been obtained.

El-Shazly *et al.* (1961a) showed that an *in vitro* fermentation of Solka Floc followed a similar rate of digestion as Solka Floc in a nylon bag which was placed in a sheep rumen. In both cases the most active phase of degradation was between 24 and 30 hr. Dehority *et al.* (1961) demonstrated that over 90% of Solka Floc was degraded between 6 and 30 hr.

In vivo work by Hale *et al.* (1947) indicated that the most active period of cellulose digestion was between 6 and 12 hr after feeding and that little

cellulose was digested between 12 and 24 hr. Le Fevre and Kamstra (1960) reported that a 48 hr in vitro fermentation of 22 different rations yielded similar digestion coefficients as standard digestion trials of the same rations, thus indicating that nearly all of the cellulose that was going to be digested was digested in 48 hr.

Some workers have suspended nylon or silk bags containing cellulose in the rumen to determine the rate of cellulose digestion. Using this technique, Lambert and Jakobson (1959) reported that the most rapid rate of decomposition of cellulose was during the first 24 hr. Lusk et al. (1962) using the nylon bag technique concluded that there was no digestion of cellulose in whole alfalfa after 36 hr. Miles (1951) demonstrated that the most active degradation of cellulose in beet pulp, alfalfa hay, and corncobs that were suspended in silk bags was during the first 36 hr of the fermentation.

The in vivo artificial rumen was used by Yadava (1962) to study the rate of breakdown of cellulose in whole alfalfa hay. Results of this experiment indicated that cellulose was rapidly degraded between 1 and 3 hr and slowly degraded from 3 to 24 hr. The breakdown between 24 and 36 hr was rapid with no digestion occurring after 36 hr.

Use of C^{14} Labeled Nutrients

In recent years there has been considerable effort expended in determining the metabolic pathways of various end-products of ruminant digestion such as acetate, propionate, and butyrate. The use of C^{14} labeled VFA has provided much useful information; however, there has been very little work with C^{14} labeled polysaccharide or a labeled natural ruminant feedstuff.

Bath and Head (1961) described a technique for labeling a small amount of perennial rye-grass with C^{14}. Hemicellulose and alpha-cellulose were extracted from rye-grass and added to an in vitro fermentation. The total labeled VFA content reached a maximum 48 hr after the addition of labeled alpha-cellulose and 24 hr after the addition of labeled hemicellulose. Samples of the artificial rumen liquor were removed at these intervals and analyzed for activity in each of the VFA.

The metabolism of C^{14} labeled glucose introduced into the rumen of a lactating cow has been reported by Otagaki et al. (1963). The specific activity of rumen VFA was greatest at 1 hr and then decreased steadily until very little activity was observed 24 hr after administration of the isotope. The specific activity of VFA removed from jugular and ruminal vein plasma reached a peak 15 min after addition of the labeled glucose. This indicated that the specific activity of rumen VFA might have been earlier than 1 hr although there were no samples of rumen fluid taken earlier to substantiate this.

Since C^{14} labeled alfalfa hay was not available commercially, a technique for its production was developed at the Kansas station by Keith et al. (1963). Preliminary trials using C^{14} labeled alfalfa have been reported by Yadava (1962). The labeled alfalfa was placed in the rumen of a cow at the time of the regular morning feeding. After administration of the isotope the cow was fed regularly at 12 hr intervals. Samples of rumen fluid, blood, urine and feces were collected at frequent intervals for 95 hr and assayed for C^{14} activity. It was recognized that subsequent feedings would result in unlabeled VFA that would dilute the specific activity of the rumen VFA. To compensate for this a dilution factor was calculated. The corrected specific activity was highest at about 40 hr and activity remained in the

VFA up to 95 hr. From this it was concluded that the most active breakdown of C^{14} labeled alfalfa hay occurred at about 40 hr and degradation continued up to 95 hr after the labeled hay reached the rumen.

Use of Polyethylene Glycol as a Marker

A water soluble reference substance is necessary to study rumen volume or rate of flow of liquid from the reticulo-rumen. Probably one of the most acceptable materials available is polyethylene glycol (PEG) with an average molecular weight of 4000. A series of publications by Hyden (1955a, 1955b, 1961) and Sperber et al. (1953) have described the characteristics of PEG and have demonstrated the use of this material. PEG was not destroyed by rumen microorganisms, was not harmful to them, and was not harmful to the cow when it was administered at the rate of 500 g per day over a period of several weeks. PEG was freely soluble in water but was not adsorbed on the rumen contents. PEG was not detected in the urine when large amounts were placed in the rumen; but when PEG was administered in the venous system it was effectively excreted by the kidneys. A turbidimetric method has been developed to provide a simple, accurate determination of PEG concentration.

Hyden (1955b) reported a 93% recovery of PEG in the feces when a single dose was administered through rumen fistula. This work was based on eight experiments with a cow and four with sheep. The amount of PEG added varied from 100 to 200 g. Unsatisfactory recovery of PEG in feces was reported by Christie and Lassiter (1958); however, the daily dosage of 15 g was probably too small for an accurate determination. Corbett et al. (1958) found that increasing the amount of PEG from 25 to 100 g gave improved but still incomplete recovery.

EXPERIMENTAL PROCEDURE

Experiment 1

Previous work by Yadava (1962) using C^{14} labeled alfalfa hay indicated that the degradation of roughage in the rumen may proceed at a much slower rate than is suggested by most workers. Since Yadava's work was based on specific activity which was corrected for dilution due to subsequent feedings, it was necessary to determine the accuracy of the dilution factor. To eliminate the dilution of specific activity due to subsequent feedings, the animal was fasted after the initial feeding of C^{14} labeled alfalfa.

Preparation of Animal. A 624 lb rumen-fistulated Jersey heifer (No. 16) was maintained for approximately 4 weeks on a daily ration of 15 lb of long alfalfa hay fed in equal amounts at 7 AM and 5 PM. On the first day of the experiment, prior to the morning feeding, a polyethylene venous catheter was installed in an external jugular vein. The regular morning ration was placed before the animal and 53.0 g of C^{14} labeled alfalfa hay, containing 3032 microcuries of activity was thoroughly mixed with the contents of the rumen. The animal was not fed again during the 72 hr experiment but she had free access to water and salt.

Collection of Samples. Samples of rumen fluid, blood, urine, and saliva were collected at frequent intervals (Appendix Table 1, 2, and 4) throughout the experiment. Immediately after collection all samples were placed in an alcohol-dry ice chest and were later transferred to a freezer. Rumen fluid samples were obtained with a syringe attached to a rubber tube extending through the cap of the cannula. A strainer attached to the terminal end of the rubber tube was maintained in a relatively fixed position in the ventral area of the rumen. Potassium oxalate solution was used as the anticoagulant

for blood samples. To prevent coagulation, the catheter was filled with a 0.1% heparin solution following the removal of each blood sample. Urine samples were obtained by catheterization of the urethra. Saliva samples were obtained from the buccal cavity with a syringe. All syringes were thoroughly washed after use to prevent contamination of subsequent samples.

Analysis of Samples. Rumen fluid samples were initially centrifuged to remove feed material. The VFA were extracted from 1 ml of the supernatant fluid by the micro steam distillation method described by Fina and Sinclair (1959). Half of the distillate was titrated with sodium hydroxide (using a phenolphthalein indicator) while nitrogen was bubbled through the distillate. This half was discarded. An equivalent amount of sodium hydroxide was added to the remaining half of distillate and this solution was evaporated to about 1 ml with a steam bath. The remaining portion was transferred to a weighed glass boat and dried under an infra-red lamp. Each glass boat was weighed again and the weight of the sodium salt of the VFA was determined by difference. One ml of water was added to the glass boat to dissolve the salt of VFA. The boat and solution were placed in a counting vial and 10 ml of phosphor solution was added.[1] The vial was cooled and radioactivity was determined with a Packard Tri-Carb, Model 314ES, Liquid Scintillation Counter. An internal standard was counted to determine the efficiency of the counter. The sample counts were corrected for efficiency and background and were expressed as disintegrations per min per mg of sodium salt of VFA, thus designating specific activity.

Blood, saliva, and urine samples were assayed for radioactivity by a

[1]Dioxone 250 ml; 2, 5-diphenyloxazole (PPO) 1.75 g; 1, 4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) 12.5 mg; and naphthalene 25 g.

modification of a method described by Herberg (1960). An internal standard was counted (Liquid Scintillation Counter) for each type of sample and activity was expressed as disintegrations per min per ml (specific activity).

Blood (0.2 ml) was dissolved in a solution containing 3 ml of 0.5 M ethanolic potassium hydroxide and 0.5 ml distilled water. Rapid mixing was essential to dissolve the blood. Ten drops of 30% hydrogen peroxide was added to change the color of the solution from a deep reddish brown to light yellow and thus reduce color quenching. Ten ml of diotol solution was added and the sample was counted.[2]

Saliva samples were centrifuged to remove feed material. Five-tenth ml of the supernatant fluid was dissolved in 3 ml of ethanolic potassium hydroxide. Ten ml of diotol was added and the sample was counted. Urine (0.5 ml) was added to 10 ml of diotol solution and the sample was counted.

Experiment 2

Dilution of specific activity by subsequent feedings was eliminated in Experiment 1; however, there may have been some dilution caused by degradation of material from previous feedings in the rumen at the start of the experiment. Experiment 2 was conducted to determine the feasibility of removing all of the material from the rumen and replacing only strained rumen fluid with the regular ration of hay.

A 1042 lb rumen-fistulated Holstein heifer (No. 20) consuming 20 lb of chopped alfalfa hay per day was used in this experiment. Half of the daily ration was fed at 7 AM and the remaining half fed at 5 PM. On the day the

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[2]Toluene 500 ml; dioxane 500 ml; methanol 300 ml; naphthalene 104 g; 2, 5-diphenyloxazole (PRO) 6.5 g; and 1, 4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) 130 mg.

experiment was initiated, prior to the morning feeding, the entire rumen contents were removed and strained through four layers of cheesecloth. The strained rumen fluid was weighed and mixed with 10 lb of ground alfalfa hay (Fineness Modulus 2.40). Two samples (approximately 400 g each) of rumen contents were collected for dry matter determination before the material was returned to the rumen. Rumen fluid samples were collected at hourly intervals for 12 hr by the method described in Experiment 1. The pH of the rumen fluid was determined each hour. Twelve hr following the start of the experiment the rumen was emptied, the contents were weighed, and samples were obtained for dry matter determination. Dry matter digestion was calculated.

Experiment 3

Since specific activity of rumen VFA was diluted by VFA from previous and subsequent feedings, it was necessary to use a dilution factor to correct specific activity. Results of Experiment 1 indicated that the dilution factor used by Yadava (1962) may have been incorrect. Consequently a different approach was sought to determine when a given amount of roughage is digested. To accomplish this, it appeared necessary to determine the amount of C^{14} activity in the total rumen VFA; however, this would necessitate knowing the total weight of rumen contents.

Polyethylene glycol[3] (PEG) has been used as an inert marker for determining rumen volume. A series of three trials was conducted to develop techniques for estimating the weight of rumen contents with PEG. In all trials PEG was added as an aqueous solution at regular intervals. The concentration

[3]Polyglycol #-4000, obtained from Dow Chemical Co., Midland, Michigan.

of PEG in the rumen was determined before and after an additional amount of PEG was placed in the rumen. The weight of rumen contents was calculated from the change in concentration due to a known amount of added PEG and compared with the known weight of rumen contents.

Trial 1. The purpose of this trial was to determine an optimum mixing time and to develop a sampling procedure. A 1042 lb rumen-fistulated Holstein heifer (No. 20) consuming 20 lb per day of chopped alfalfa hay was used. Equal amounts of daily ration were fed at 7 AM and 5 PM. Three hr following the morning feeding the entire rumen contents were removed, weighed, and returned to the rumen. Fifty g of PEG contained in a beaker was poured into the rumen. The rumen contents were vigorously hand mixed for 3 min and a sample of rumen contents was collected. Approximately 400 g was removed from the rumen by hand and placed in a glass tray. Several small portions of this material were removed by hand and placed in a 4 oz jar. Samples were also collected 6, 9, and 12 min after the addition of PEG. The rumen contents were mixed constantly during this period except for the time that samples were being removed.

Four hr following the initial addition of PEG, the rumen contents were removed, weighed, and returned to the rumen. A sample of rumen contents was collected and 50 g of PEG was added. Samples of rumen contents were collected 3, 6, 9, and 12 min after the addition of PEG. The rumen contents were vigorously hand mixed between sample removal. The concentration of PEG in the samples was determined by the method described by Hyden (1955a).

Trial 2. The purpose of this trial was to evaluate a different sampling procedure and determine the effect of quantity of PEG on the accuracy of the method. The same animal (No. 20) and ration used in Trial 1 was used in this trial. Prior to the morning feeding the rumen contents were removed and

strained through four layers of cheesecloth. The residue remaining in the cheesecloth was discarded. The strained rumen fluid was weighed, mixed with 10 lb of ground alfalfa hay (Fineness Modulus 2.40), and returned to the rumen. Fifty g of PEG was poured into the rumen using a funnel connected to rubber tubing. The terminal end of the rubber tubing was moved throughout the rumen while the PEG solution was being added to prevent the concentration of PEG in any area. The entire rumen contents were mixed for 8 min and a sample was collected in the following manner. Ten samples of about 25 g each were taken from different parts of the rumen and placed in a weighed gallon sampling jar. The jar was weighed again and the weight of the sample was determined by difference. A known dilution of the sample was made with water and the sample was brought to the laboratory for assay. This sampling procedure was used in all subsequent trials.

Four hr after the start of the trial the rumen contents were removed, weighed, and returned to the rumen. A sample of rumen contents was then obtained and 10 g of PEG was added to the rumen after sampling. The rumen contents were mixed for 8 min and another sample was collected. The concentration of PEG in the samples was determined by the method described by Hyden (1955a).

Trial 3. The purpose of this trial was to demonstrate that the weight of rumen contents could be accurately estimated with PEG following procedures developed in the first two trials. A 627 lb rumen-fistulated Jersey heifer (No. 16) was used in this trial. She was maintained on a daily ration of 14 lb of chopped alfalfa hay fed in equal amounts at 7 AM and 5 PM. On the day of the trial, before the morning feeding, the rumen contents were removed and strained through four layers of cheesecloth. The strained rumen fluid

was weighed, mixed with 7 lb of ground alfalfa hay (Fineness Modulus 2.20), and returned to the rumen. Forty g of PEG was distributed in the rumen with a funnel and rubber tubing, and the rumen contents were hand mixed for 5 min. Triplicate samples of rumen contents were obtained. The coarse material was removed and discarded from one of the samples by straining the sample through four layers of cheesecloth.

Four and 8 hr after the start of the trial the rumen contents were removed, weighed, and returned to the rumen. Before an additional 40 g of PEG was added, one sample was obtained from the container while the rumen contents were out of the rumen, a second sample was obtained from within the rumen, and a third sample was obtained from within the rumen and strained through four layers of cheesecloth. After the addition of 40 g of PEG, the mixing and sampling procedure was similar to that following the initial addition of PEG at 8 AM.

Experiment 4

Preparation of Animal. The purpose of this experiment was to determine the specific activity of rumen VFA under conditions in which there would be no dilution due to previous or subsequent feedings. In addition, total activity was determined and compared with specific activity. The same animal (No. 16) and ration used in Trial 3 of Experiment 3 were used in this experiment. Before the morning feeding the rumen contents were removed and strained through four layers of cheesecloth. The residue remaining in the cheesecloth was discarded. The strained rumen fluid was weighed, mixed with 7 lb of ground alfalfa hay (Fineness modulus 2.20), and returned to the rumen. C^{14} labeled alfalfa hay (47.5g) containing 3152 microcuries of activity was

placed in the rumen. The animal was fasted for 48 hr but had access to water and salt. Ground alfalfa hay (3.5 lb) was placed in the rumen 48 hr after the start of the trial.

Collection of Samples. Samples of rumen fluid were obtained by the method described in Experiment 1 and immediately placed in an alcohol-dry ice chest. At frequent intervals (Table 6) 40 g of PEG was distributed throughout the rumen and the rumen contents were hand mixed for 5 min. Samples of rumen contents were obtained for PEG determination before and after PEG was poured into the rumen.

Each time that PEG was added a composite sample of rumen contents was collected by combining four samples (about 20 g each) that were approximately equal in size and dry matter content, and taken from different areas of the rumen. This sample was immediately placed in an alcohol-dry ice chest.

Analysis of Samples. The rumen fluid samples were assayed by the same procedure used in Experiment 1. The activity was expressed as disintegrations per min per mg of salt of VFA (specific activity). The concentration of PEG in the samples was determined by the method of Hyden (1955a).

The composite samples of rumen contents were assayed in the following manner. Duplicate dry matter determinations were made on each sample. Approximately a third of each dry matter sample was removed and saved for dry combustion. Cellulose was removed from the remaining dry matter by the Crampton-Maynard (1938) method. Weighed samples of cellulose and rumen dry matter were assayed for C^{14} activity by a dry combustion technique described by Kelley et al. (1961). The phosphors solution of Jeffay and Alvarez (1961) was used for counting the samples. The C^{14} activity was expressed as disintegration per min per mg of sample (specific activity).

Experiment 5

Since C^{14} labeled alfalfa hay was produced under environmental conditions which differed from those which existed during the production of field cured hay, it was necessary to determine whether these two hays would be fermented at the same rate by rumen microorganisms. Four mixtures containing different proportions of labeled and unlabled hay (Table 1) were fermented in vitro. The specific activity of the VFA produced at various intervals of time from each of the four mixtures was used as an index of rate of fermentation of the two hays. The ratios of labeled to unlabeled hay in the substrate varied from the approximate ration used in Experiment 4 (1 to 63) to a substrate containing labeled hay only. The in vitro technique of Baumgardt et al. (1962) was used for this experiment.

Table 1. Substrate for in vitro fermentation

Mixture	Ratio of labeled to unlabeled hay	Labeled hay	Unlabeled hay
		mg	mg
I	1:63	31.3	1968.7
II	1:21	91.0	1909.0
III	1:7	250.0	1750.0
IV	1:0	2000.0	0.0

Duplicate samples were fermented for each mixture. A 3 ml sample of rumen fluid was removed from each fermentation flask under anaerobic conditions at 2, 4, 6, 8, 12, 18, and 24 hr and immediately placed in a freezer. The rumen fluid samples were assayed by a procedure described in Experiment 1. The specific activity at each sampling time was determined and expressed as disintegrations per min per mg of salt of VFA (specific activity).

RESULTS

Experiment 1

The specific activity of rumen VFA, blood, saliva, and urine assayed in duplicate is shown in Appendix Tables 1, 2, 3, and 4 respectively. The production of VFA at various times after feeding is shown in Fig. 1 and Appendix Table 1. The specific activity of rumen VFA, blood, saliva, and urine that were collected at various times following administration of the isotope is illustrated in Fig. 2, 3, 4, and 5 respectively.

The production of VFA (Fig. 1) increased rapidly after feeding and reached a peak at about 6 hr. After 6 hr. the level of VFA gradually decreased throughout the remainder of the experiment. The peak production of VFA occurred about 2 or 3 hr later than would normally be expected and the production of VFA was higher at 12 hr than at 0 hr. The delayed peak may have been a result of the animal's not consuming all of her ration until about 8 hr after the start of the experiment.

The specific activity of rumen VFA (Fig. 2) increased rapidly following administration of the isotope with the peak activity occurring at 4 hr. The activity diminished rapidly for the next 8 hr. There was little change in specific activity from 12 hr until the end of the experiment.

The specific activity of urine (Fig. 5) followed a similar pattern as the rumen VFA; however, the rise and decline trailed the activity of the rumen VFA by about 2 hr. The urine activity diminished gradually from 12 hr until the end of the experiment although there was a second small peak at 42 hr.

The specific activity of saliva (Fig. 4) was erratic during the first

few hours. This may have been due to poor sampling. Generally the activity in saliva was high during the first few hours and gradually diminished up to 72 hr.

The specific activity of blood (Fig. 3) followed an entirely different pattern than rumen VFA, urine, or saliva. Specific activity was low during the first few hours and increased to a peak at about 10 hr. Specific activity decreased from 12 to 24 hr and then gradually increased from 24 to 72 hr. The specific activity at the end of the experiment was slightly higher than the early peak at 10 hr.

Experiment 2

This experiment was conducted to determine whether hay placed in an empty rumen with strained rumen fluid would be digested normally. The pH of rumen fluid sampled at hourly intervals is shown in Table 2.

Table 2. pH of rumen fluid at various time intervals following the complete removal of rumen contents and reintroduction of strained rumen fluid and a normal ration of hay.

Hours	0	1	2	3	4	5	6	7	8	9	10	11	12
pH	7.2	6.9	6.7	7.0	7.0	7.1	6.9	7.0	7.1	7.2	7.2	7.3	7.2

The pH of rumen fluid followed a relatively normal pattern. The pH dropped after the start of the trial and reached the lowest level at 2 hr. The pH reached the level found at the start of the trial at 9 hr and then remained at a fairly constant level until the end of the experiment. Although the magnitude of the drop in pH at 2 hr was not so great as would normally be expected, it was considered normal for the conditions of this experiment.

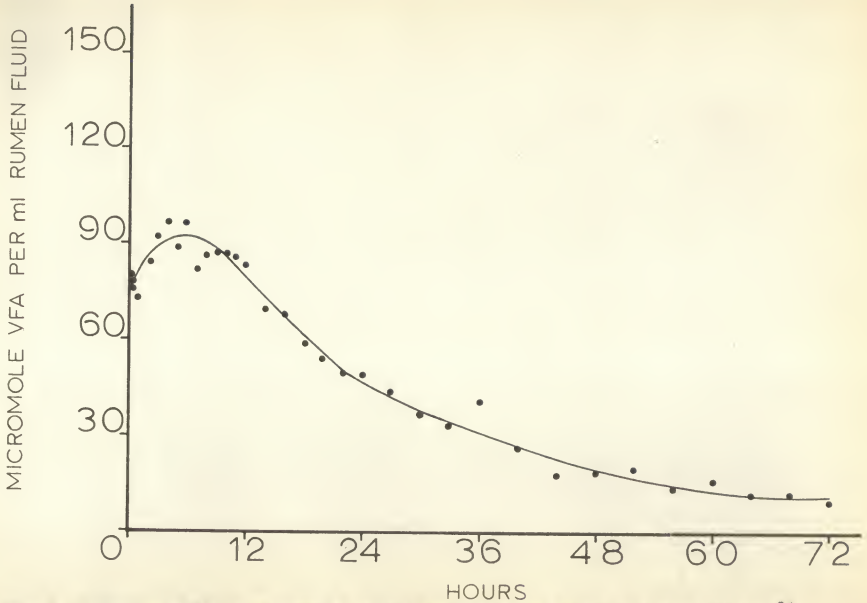

Fig. 1. Production of VFA in the rumen of heifer fasted after one feeding of alfalfa hay and C^{14} labeled alfalfa.

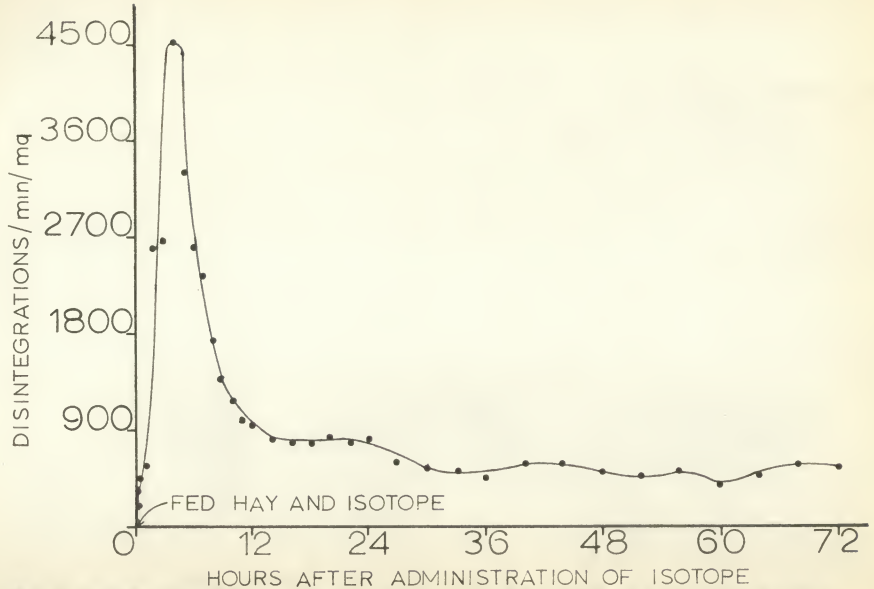

Fig. 2. Specific activity (disintegrations per min per mg) of Na salt of VFA in the rumen of heifer fasted after one feeding of alfalfa hay and C^{14} labeled alfalfa.

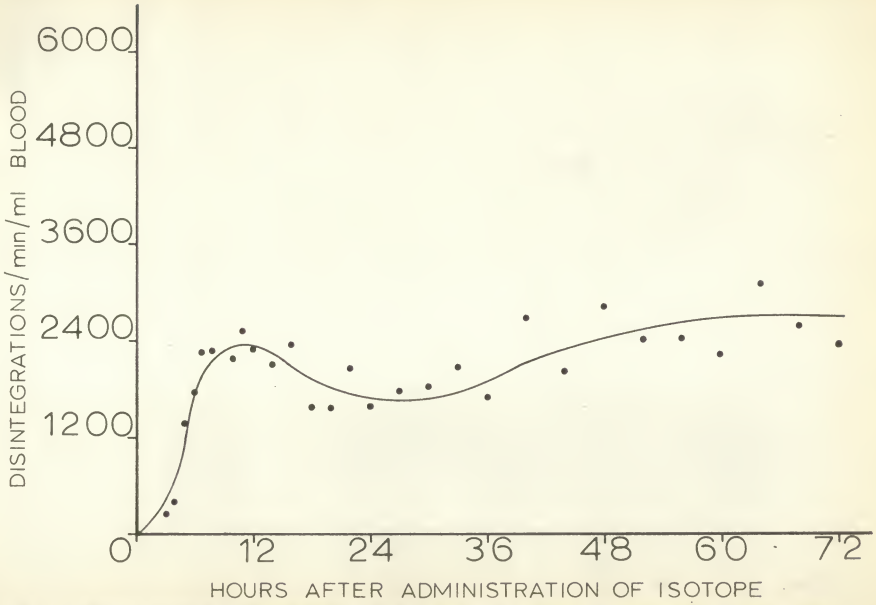

Fig. 3. Specific activity (disintegrations per min per ml) of jugular blood from heifer fasted after one feeding of alfalfa hay and C^{14} labeled alfalfa.

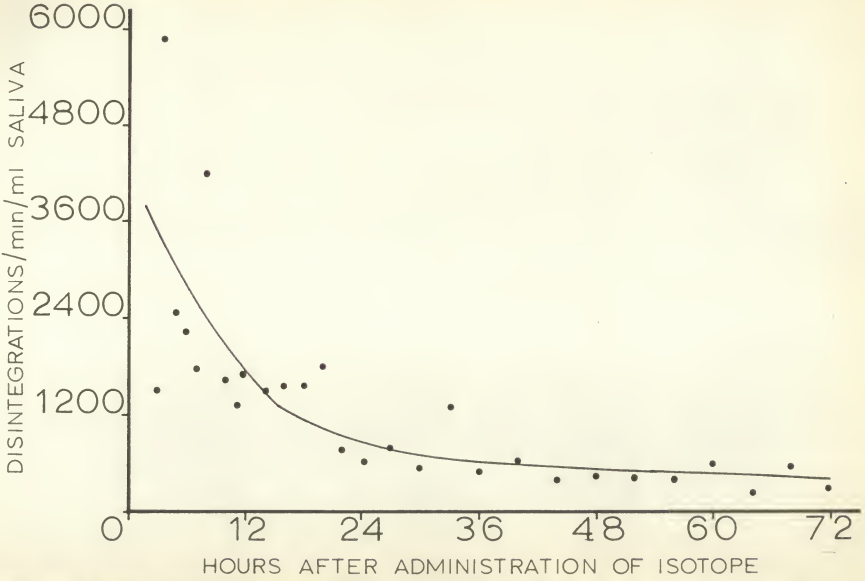

Fig. 4. Specific activity (disintegrations per min per ml) of saliva from heifer fasted after one feeding of alfalfa hay and C^{14} labeled alfalfa.

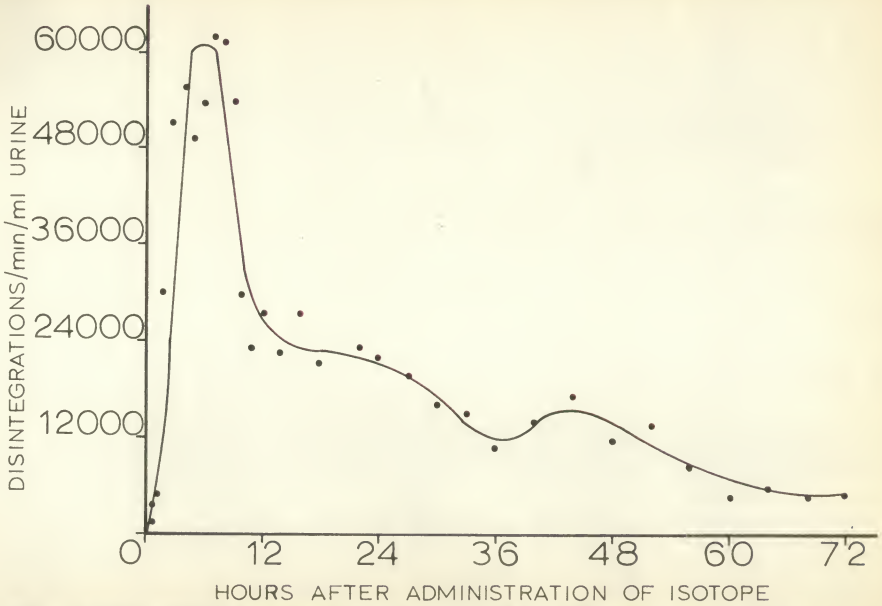

Fig. 5. Specific activity (disintegrations per min per ml) of urine from heifer fasted after one feeding of alfalfa hay and C^{14} labeled alfalfa.

At the start of the trial the rumen contained 10 lb of dry matter which comprised 16% of the total rumen contents. Twelve hr later the rumen contained 6.1 lb of dry matter which comprised 6.4% of the total rumen contents. From this it was calculated that 39% of the dry matter present at the start was removed in 12 hr. The trial was stopped at 12 hr because it was evident from dry matter removal and pH that digestion was proceeding at a normal rate. Also the odor of rumen fluid was normal and gas bubbles were numerous which suggested an active fermentation.

Experiment 3

Trial 1. The results of this trial using PEG as a marker to estimate the weight of rumen contents are shown in Table 3. Increasing the mixing time appeared to increase the accuracy at 11:30 AM; however, this trend was not evident at 4:00 PM. The sampling procedure of removing several samples from the rumen by hand and taking a sample of the composite for analysis was not satisfactory because it was difficult to obtain a sample that had the same proportion of dry matter as that of the rumen contents. It appeared that improved sampling was necessary to estimate accurately the weight of rumen contents.

Trial 2. Results of this trial are shown in Table 4. An improved sampling technique described in the Experimental Procedure was used. The calculated weight of rumen contents was very close to the known weight at 8:00 AM.

At 12 noon a smaller quantity of PEG was used than at 8AM. It was apparent that the smaller amount of PEG adversly affected the accuracy of the technique. For example, at 8:00 AM the expected change in concentration

Table 3. Estimation of weight of rumen contents using PEG marker (Experiment 3, Trial 1)

Sample no.[1]	Mixing time	PEG concn in sample	PEG concn in rumen[2]	Change in concn[3]	Calculated wt rumen contents	% of known wt of rumen contents[4]
	min	mg/g	mg/g	mg/g	g	
			11:30 AM after PEG added			
1A	3	0.072	1.08	1.08	46296	65.2
1B	3	0.076	1.14	1.14	43859	61.7
2A	6	0.061	0.92	0.92	54348	76.5
2B	6	0.064	0.96	0.96	52083	73.3
3A	9	0.049	0.74	0.74	67568	95.1
3B	9	0.058	0.87	0.87	57471	80.9
4A	12	0.050	0.75	0.75	66667	93.8
4B	12	0.051	0.77	0.77	64935	91.4
			4:00 PM before PEG added			
5A		0.044	0.66			
5B		0.041	0.62			
			4:00 PM after PEG added			
6A	3	0.102	1.53	0.87	57471	96.3
6B	3	0.102	1.53	0.91	54945	92.0
7A	6	0.108	1.62	0.96	52083	87.2
7B	6	0.105	1.58	0.96	52083	87.2
8A	9	0.100	1.50	0.84	59524	99.7
8B	9	0.100	1.50	0.88	56818	95.2
9A	12	0.093	1.40	0.74	67568	113.2
9B	12	0.092	1.38	0.76	65789	110.2

[1]A and B designate duplicates assayed on two different days.

[2]Sample initially diluted 1:3 with water. Method used 4 ml sample, 5 ml reagents, and 11 ml water. Total dilution 1:15.

[3]Change in concentration due to addition of 50 g PEG.

[4]Calculated wt expressed as % of known wt of rumen contents. Known wt at 11:30 AM was 71051 g. Known wt at 4:00 PM was 59701 g.

Table 4. Estimation of weight of rumen content from two different quantities of PEG (Experiment 3, Trial 2)

Before PEG added		After PEG added		Change in concn[2]	Calculated wt rumen contents	% of known wt of rumen contents[3]
Sample no.[1]	Concn in rumen	Sample no.[1]	Concn in rumen			
	mg/g		mg/g	mg/g	g	
			8:00 AM			
	0.	1A	1.60	1.60	31250	98.1
	0.	1B	1.58	1.58	31646	99.3
	0.	1C	1.60	1.60	31250	98.1
			12:00 noon			
2A	0.86	3A	1.16	0.30	33333	80.3
2B	0.86	3B	1.16	0.30	33333	80.3
2C	0.87	3C	1.15	0.29	34483	83.1

[1] A, B, and C are triplicate samples assayed at the same time.

[2] Change in concentration due to the addition of 50 g at 8:00 AM and 10 g at 12:00 noon.

[3] Calculated wt expressed as % of known wt of rumen contents. Known wt were 31865 g at 8:00 AM and 41505 g at 12:00 noon.

based upon the known weight of rumen contents and 50 g of added PEG was 1.57 mg/g. Sample 1A differed from the theoretical change in concentration by 0.03 mg/g but the error in estimating the weight of rumen contents was only 1.9%. The theoretical change in concentration at 12:00 noon was .24 mg/g. Samples 2A and 3A indicated a change in concentration that was in error by .06 mg/g. While this error was only twice as large as that at 8:00 AM, the error in estimating the weight of rumen contents was ten times larger.

The results of this trial indicated that a satisfactory estimation of the weight of rumen contents could be made if PEG was added at a rate of at least 1 g/20 lb body weight of the animal.

Trial 2. The object of this trial was to demonstrate that PEG could be used to estimate accurately the weight of rumen contents. The results are shown in Table 5. Duplicate samples of rumen contents agreed well. There appeared to be a greater day to day variation in the assay procedure than between duplicate samples. One sample of rumen contents was strained through cheesecloth each time PEG was added to determine how accurately the weight of rumen contents could be determined from strained rumen fluid. The results indicated that the weight of rumen contents could not satisfactorily be estimated from a sample of strained rumen fluid but could be estimated with accuracy when the whole rumen contents were sampled.

Experiment 4

The production of VFA, specific activity of rumen VFA, and total activity in the rumen VFA are shown in Appendix Table 6 and graphically illustrated in Fig. 6, 7, and 8 respectively. Specific activity (Fig.7) increased rapidly following administration of the isotope, reached a peak at 8 to 10 hr, and then gradually declined until 48 hr. When additional feed was placed in the rumen at 48 hr VFA production (Fig. 6) increased rapidly and there was a corresponding decrease in specific activity (Fig. 7). The total activity in rumen VFA (Fig. 8) was unaffected by the additional feed.

PEG was used to estimate the weight of rumen contents at regular intervals throughout the trial. Duplicate determinations of the weight of rumen contents are shown in Table 6. These data were used for calculating the total activity in rumen VFA. The total amount of dry matter and cellulose in the rumen at regular intervals throughout the trial is shown in Table 7.

The specific activity of cellulose and rumen dry matter is shown in Appendix Table 7. The total activity in cellulose and dry matter remaining in

Table 5. Estimation of weight of rumen contents with PEG when sample consisted of either whole rumen contents or strained rumen fluid (Experiment 3, Trial 3)

Before PEG added		After PEG added		Change in concn[2]	Calculated wt rumen contents	% of known wt of rumen contents[3]
Sample no.[1]	Concn in rumen	Sample no.[1]	Concn in rumen			
	mg/g		mg/g	mg/g	g	
			8:00 AM			
	0.	1A[a]	1.55	1.55	25806	94.0
	0.	1B[a]	1.49	1.49	26846	97.7
	0.	2A[a]	1.53	1.53	26230	95.5
	0.	2B[a]	1.49	1.49	26846	97.7
	0.	3A[b]	1.90	1.90	21053	76.6
	0.	3B[b]	1.89	1.89	21164	77.1
			12:00 noon			
4A[c]	0.85	7A[a]	2.20	1.37	29197	94.6
4B[c]	0.78	7B[a]	1.98	1.23	32520	105.3
5A[a]	0.83	8A[a]	2.17	1.34	29851	96.7
5B[a]	0.75	8B[a]	2.03	1.28	31250	101.2
6A[b]	0.87	9A[b]	2.30	1.47	27211	88.1
6B[b]	0.82	9B[b]	2.17	1.42	28169	91.2
			4:00 PM			
10A[c]	1.89	13A[a]	3.40	1.51	26490	107.3
10B[c]	1.68	13B[a]	3.12	1.47	27211	110.2
11A[a]	1.89	14A[a]	3.50	1.61	24845	100.6
11B[a]	1.65	14B[a]	3.22	1.57	25478	103.2
12A[b]	1.98	15A[b]	3.72	1.83	21858	88.5
12B[b]	1.91	15B[b]	3.36	1.71	23392	94.7

[1]A and B designate duplicates assayed on two different days.

[2]Change in concentration due to the addition of 40 g PEG. Samples 5 and 11 were used in calculating change in concentration at 12:00 noon and 4:00 PM respectively.

[3]Calculated wt expressed as % of known wt of rumen contents. Known wt were 27467 g at 8:00 AM, 30872 g at 12:00 noon, 24697 g at 4:00 PM.

[a]Samples of rumen contents taken from rumen.

[b]Samples taken from rumen and strained through 4 layers of cheesecloth.

[c]Samples of rumen contents taken from container when rumen contents were removed to be weighed.

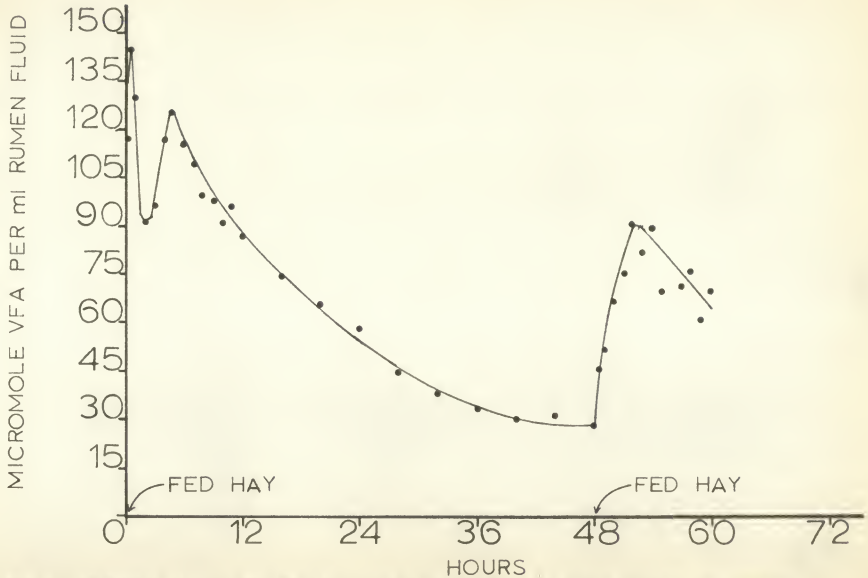

Fig. 6. Production of rumen VFA after introducing alfalfa hay, C^{14} labeled alfalfa, and strained rumen fluid into an empty rumen at 0 hr and adding alfalfa hay at 48 hr.

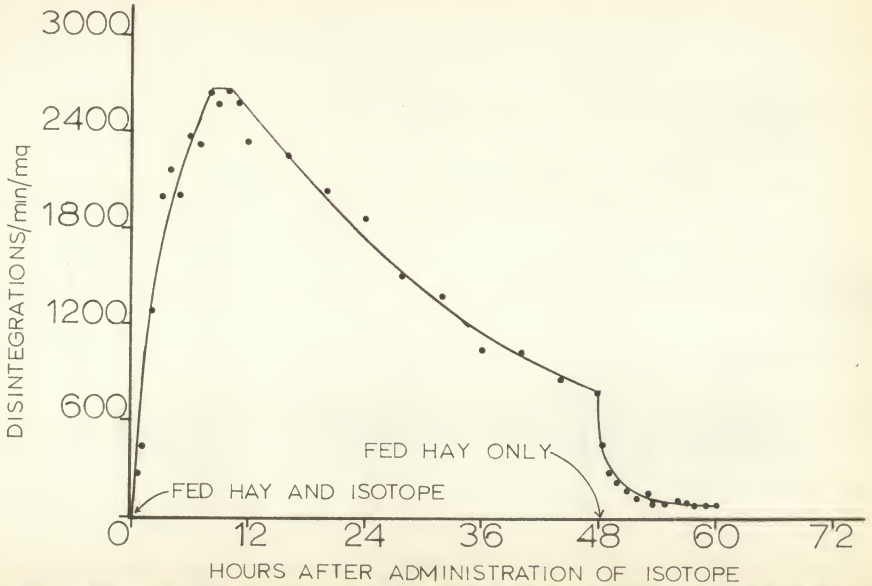

Fig. 7. Specific activity (disintegrations per min per mg) of Na salt of rumen VFA after introducing alfalfa hay, C^{14} labeled alfalfa, and strained rumen fluid into an empty rumen at 0 hr and adding alfalfa hay at 48 hr.

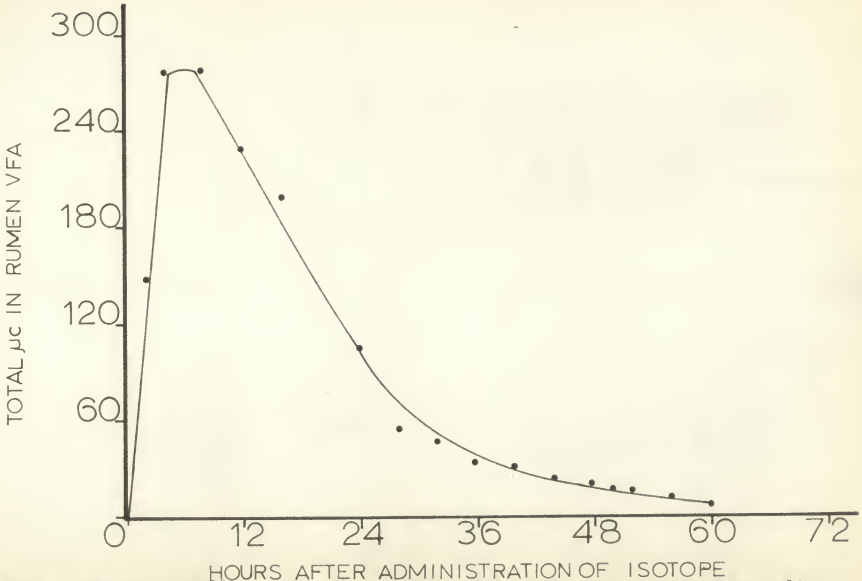

Fig. 8. Microcuries of C^{14} in total rumen VFA (total activity) after introducing alfalfa hay, C^{14} labeled alfalfa, and strained rumen fluid into an empty rumen at 0 hr and adding alfalfa hay at 48 hr.

Table 6. Estimation of weight of rumen contents with PEG at regular intervals throughout Experiment 4

Hour	Before PEG added: Sample no.	Before PEG added: Concn in rumen	After PEG added: Sample no.	After PEG added: Concn in rumen	Change in concn[1]	Calculated wt rumen contents
		mg/g		mg/g	mg/g	g
2			1A	1.22	1.22	32787
			1B	1.18	1.18	33898
4	2A	1.18	3A	2.56	1.38	28986
	2B	1.24	3B	2.56	1.32	30303
8	4A	2.12	5A	3.50	1.38	28986
	4B	1.96	5B	3.43	1.47	27211
12	6A	3.05	7A	4.60	1.55	25806
	6B	3.00	7B	4.30	1.30	30769
16	8A	4.55	9A	5.90	1.35	29630
	8B	4.50	9B	5.78	1.28	31250
24	12A	7.15	13A	9.00	1.85	21622
	12B	6.90	13B	8.75	1.85	21622
28	14A	7.30	15A	9.40	2.10	19048
	14B	8.00	15B	9.80	1.80	22222
32	16A	9.74	17A	11.52	1.78	22472
	16B	9.35	17B	11.33	1.98	20202
36	18A	8.73	19A	10.45	1.72	23256
	18B	9.02	19B	10.56	1.54	25974
40	20A	9.90	21A	11.55	1.65	24242
	20B	9.63	21B	11.22	1.59	25157
44	22A	10.34	23A	12.10	1.76	22727
	22B	11.10	23B	12.83	1.73	23121
48	24A	11.94	25A	13.64	1.70	23529
	24B	11.44	25B	13.20	1.76	22727
50	26A	9.90	27A	11.33	1.43	27972
	26B	9.02	27B	10.67	1.65	24242
52	28A	9.57	29A	10.62	1.05	38095
	28B	9.13	29B	10.12	.99	40404
56	30A	9.02	31A	10.01	.99	40404
	30B	9.02	31B	10.12	1.10	36364
60	32A	7.87	33A	9.02	1.15	34783
	32B	7.59	33B	8.75	1.16	34483

[1] 40 g PEG were added each time samples were taken.

Table 7. Total weight of dry matter and cellulose in the rumen at regular intervals throughout Experiment 4

Hour	Sample no.	Wt rumen contents[1]	% DM	Wt DM	Cellulose % of DM	Wt cellulose
		g		g		g
0		16392		3366		1079
2	3A	33343	10.92	3641	29.73	1083
	3B	"	10.90	3634	30.35	1103
4	4A	29644	10.89	3228	31.51	1017
	4B	"	11.32	3356	33.46	1123
8	5A	28099	8.69	2442	30.21	738
	5B	"	8.91	2504	31.23	782
12	6A	28288	8.61	2436	29.97	730
	6B	"	8.39	2373	29.73	706
16	7A	30443	8.25	2512	27.65	694
	7B	"	7.94	2417	26.59	643
24	9A	21622	7.96	1721	25.33	436
	9B	"	7.76	1678	25.23	423
28	10A	20635	7.69	1587	25.62	407
	10B	"	7.40	1527	24.75	378
32	11A	21337	7.53	1607	24.69	397
	11B	"	7.70	1643	25.32	416
36	12A	24615	7.71	1898	29.39	558
	12B	"	7.50	1846	28.97	535
40	13A	24242	8.43	2044	29.43	601
	13B	"	8.32	2017	30.40	613
44	14A	22924	9.26	2123	31.02	659
	14B	"	8.74	2003	30.15	604
48	15A	23128	9.34	2160	30.51	659
	15B	"	9.44	2183	31.21	681
50	16A	26107	11.14	2908	31.45	915
	16B	"	11.60	3028	31.60	957
52	17A	39250	10.45	4102	31.29	1283
	17B	"	10.62	4168	32.22	1343
56	18A	38384	9.59	3681	31.14	1146
	18B	"	9.71	3727	31.32	1167
60	19A	34633	9.30	3220	32.21	1037
	19B	"	9.55	3308	32.49	1075

[1]See Table 6.

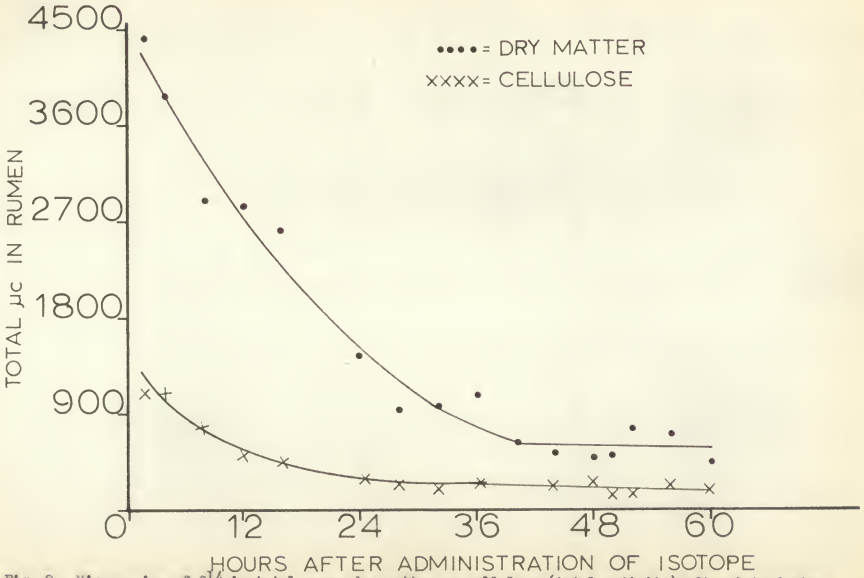

Fig. 9. Microcuries of C^{14} in total rumen dry matter or cellulose (total activity) after introducing alfalfa hay, C^{14} labeled alfalfa, and strained rumen fluid into an empty rumen at 0 hr and adding alfalfa hay at 48 hr.

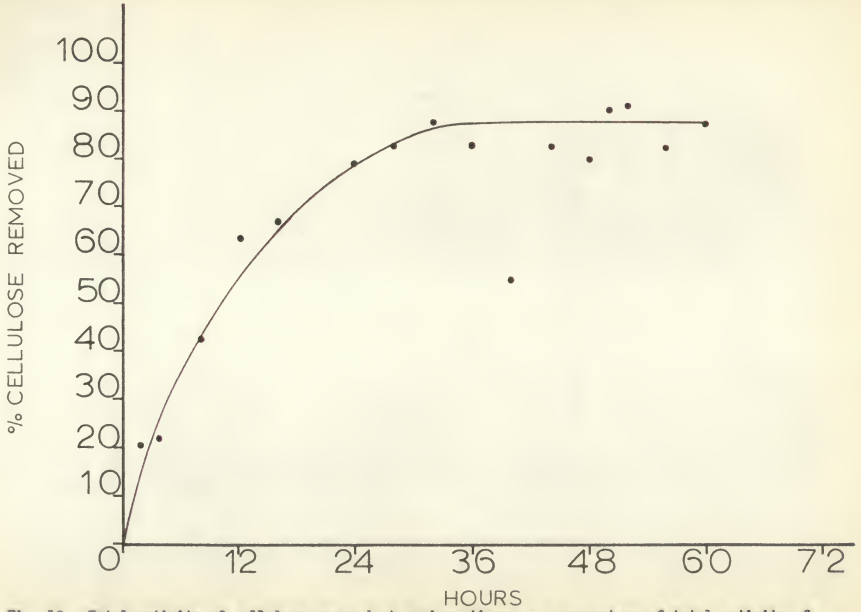

Fig. 10. Total activity of cellulose removed at various times as a percentage of total activity of cellulose portion of hay fed.

the rumen is shown in Appendix Table 7 and graphically illustrated in Fig. 9. The total activity was calculated by multiplying the specific activity of cellulose or dry matter (Appendix Table 7) by the weight of cellulose or dry matter in the rumen (Table 7) at each sampling time. It is evident from data presented in Fig. 9 that the most active period of degradation of dry matter and cellulose was the first 12 hr after the labeled hay was introduced into the rumen. There was little removal of cellulose or dry matter after 36 hr.

The removal of cellulose is shown in Fig. 10. This was based on 1370 microcuries of C^{14} activity in the cellulose at the start of the trial. The percentage of cellulose removed at each sampling time was calculated by dividing the activity in cellulose removed (Appendix Table 7) from the rumen by the total activity in the cellulose at the start of the trial.

Experiment 5

The specific activity of VFA is shown in Table 8. It was impossible to compare the specific activity of all the treatments at each sampling time because a different amount of activity was present at the start of the fermentation so the activity at each sampling time was expressed as a percentage of the activity of the same fermentation at 24 hr. A comparison of these percentages reveals that the different treatments followed a similar increase in specific activity at each sampling time. It was concluded that the labeled and unlabeled hays were digested at a similar rate.

Table 8. Specific activity of VFA production in vitro of several ratios of labeled to unlabeled substrate

Hr[1]	Duplicate	Specific activity (DPM/mg)				% of specific activity at 24 hr			
		I[2]	II	III	IV	I	II	III	IV
2	A	510	968	2488	27810	35.7	38.5	27.8	36.8
	B	439	856	3549	23715	38.8	30.8	38.1	31.2
4	A	972	1502	4570	48677	68.1	59.8	51.1	64.4
	B	842	1949	6492	46122	74.4	70.2	69.7	60.8
6	A	994	1267	5743	55476	69.7	78.3	64.2	73.4
	B	822	1931	6693	51472	72.7	69.6	71.9	67.8
8	A	1127	2017	6558	62391	79.0	80.3	73.3	82.5
	B	884	2028	6043	62112	78.2	73.1	64.9	18.8
12	A	1024	1864	6484	68073	71.8	74.2	72.5	90.0
	B	1130	2573	6865	55872	99.9	92.7	73.7	73.6
18	A	1311	2587	8880	69990	91.9	103.0	99.3	92.5
	B	1140	2115	8403	66153	100.8	76.2	90.2	87.2
24	A	1427	2512	8943	75627	100.0	100.0	100.0	100.0
	B	1131	2775	9312	76898	100.0	100.0	100.0	100.0

[1]Hr after start of fermentation.

[2] I Ratio of labeled to unlabeled hay 1:63.
II Ratio of labeled to unlabeled hay 1:21.
III Ratio of labeled to unlabeled hay 1:7.
IV Ratio of labeled to unlabeled hay 1:0.

DISCUSSION

Previous work by Yadava (1962) and Experiments 1 and 4 of this thesis were based on the principle that a small amount of C^{14} labeled alfalfa hay placed in the rumen would become intimately mixed with the unlabeled hay fed at the same time. The degradation of the labeled hay would be expected to be representative of the degradation of the total roughage fed. The rate of breakdown or digestion was based on the specific activity of the rumen VFA at

varying intervals of time after administration of the isotope. It was postulated that the level of specific activity in the rumen VFA at a particular time would correspond to the breakdown of roughage at that time.

If the labeled hay was actually thoroughly mixed with unlabeled hay, it seems that the labeled and unlabeled roughages would break down and produce VFA at a corresponding rate. Any increase in rate of digestion of labeled hay would be accompanied by a corresponding increase in rate of digestion of unlabeled hay. This would result in a rapid increase in the specific activity of the rumen VFA when the labeled hay starts to break down and then remain at a constant level until some outside source of VFA dilutes the specific activity. The outside source of VFA could be produced from breakdown of roughage already in the rumen due to previous feedings or from new roughage fed after administration of isotope. If this reasoning is correct, it seems that the specific activity might remain at a given level over an extended period of time, even though the rate of digestion was changing during that same time period.

Dilution of specific activity by VFA resulting from subsequent feedings was eliminated in Experiment 1 because the animal was fasted. As expected, the specific activity of the rumen VFA rapidly increased during the first few hours after adding the labeled hay. However, instead of remaining at a high level, the specific activity dropped rapidly and at 12 hr it leveled off and remained at a constant level throughout the remainder of the experiment. The fall in specific activity may have been due to dilution resulting from breakdown of cellulose remaining in the rumen from previous feedings. It is possible that the addition of new feed stimulated digestion of feed already in the rumen. Another possible reason for the drop in specific activity was

that the animal did not readily eat all of her unlabeled hay ration until about 8 hr after administration of the isotope. The breakdown of this hay would dilute the specific activity of the VFA. In this particular trial one could not necessarily assume that the labeled and unlabeled hays were digested at the same rate since much of the unlabeled hay was not even in the rumen at the time the labeled hay started to break down.

If the theory advanced by Yadava (1962) that the level of specific activity of rumen VFA at a particular time corresponds to the breakdown of roughage at that time is correct, the results of Experiment 1 suggest that maximum breakdown of alfalfa hay occurred during the first 12 hr. These findings are not in agreement with the conclusions of Yadava (1962) that the maximum breakdown of roughage occurred at about 40 hr. The specific activity of urine and saliva (Experiment 1) followed the same general pattern as rumen VFA to give further evidence that maximum breakdown of roughage occurs during the first 12 hr.

The specific activity of blood peaked at about 8 hr and a second peak appeared near the end of the experiment. Since it was not known in what form the C^{14} existed in blood, it is difficult to draw any conclusions from the second peak of activity. It might be conjectured that C^{14} was being metabolized for a considerable period of time from the C^{14} body pool.

A study of Fig. 2 (Experiment 1) reveals that there was very little change in the specific activity of rumen VFA from 12 to 72 hr. According to the previously mentioned supposition, this would suggest that the labeled hay was digested at a constant rate during this period. The possibility that this was happening over a 60 hr period appears remote. A more logical explanation would be that an equilibrium had been reached between the labeled VFA

and the unlabeled VFA produced from previous feedings and the unlabeled hay fed. If this were the case, it suggests that the level of specific activity of rumen VFA would not necessarily reflect the amount of breakdown of a roughage at any particular time. To pursue this line of reasoning, a trial was conducted in which there was no dilution due to previous or subsequent feedings.

Results of Experiments 2 and 3 showed that digestion could proceed in a somewhat normal fashion and the weight of rumen contents could be accurately determined when all of the roughage in the rumen was discarded and the strained rumen fluid was replaced along with a small amount of roughage. Using these techniques, Experiment 4 was conducted and the specific activity and total activity of the rumen VFA was determined.

Under the conditions of Experiment 4, the specific activity of rumen VFA was expected to increase when labeled hay started to be digested and remain at that level until additional unlabeled hay was fed. However, the specific activity leveled off for only a few hours and then started to decrease gradually. The only explanation for this decrease was that some hay and wood shavings (used for bedding) were consumed by the cow despite efforts to prevent her from eating. It was not known how much material was consumed but it is possible that enough was eaten and digested to dilute the specific activity. A comparison of Fig. 2 (Experiment 1) and Fig. 7 (Experiment 4) suggests that some VFA are produced from material already in the rumen and dilute the specific activity since the specific activity shown in Fig. 2 dropped much more rapidly than that shown in Fig. 7.

Dilution of specific activity by subsequent feedings was clearly demonstrated in Experiment 4. When feed was placed in the rumen at 48 hr the

production of VFA immediately increased (Fig. 6) and the specific activity of the rumen VFA showed a corresponding decrease (Fig. 7).

Since the level of specific activity of rumen VFA resulting from the breakdown of labeled hay was affected by previous and subsequent feedings, it did not appear that specific activity was a satisfactory measure of the breakdown of labeled hay. A more logical method for determining when the labeled hay is broken down is to determine the amount of activity in the total rumen VFA at regular intervals after administration of the isotope. This was done by determining the activity in a given weight of rumen contents, determining the total weight of rumen contents, and calculating the activity in the entire rumen VFA.

Immediately after administration of the isotope there was activity in the rumen; however, activity did not appear in the rumen VFA until the labeled hay started to break down. One would expect that as more labeled hay was digested a greater amount of activity would be present in the rumen VFA. Since no information on the rate of absorption of VFA was available, it would not be entirely correct to assume that the total amount of activity in the rumen VFA at a particular time reflected the amount of digestion at that time. However, it appears that total activity would in general reflect the rate of digestion of labeled hay.

The total activity graph (Fig. 8) looked very much like the specific activity graph (Fig. 7); however, the similarity in shape of the two should not be construed as conveying the same meaning. The shape of the curves at 48 hr clearly illustrates the difference between the graphs. While the feeding of unlabeled hay diluted the specific activity, it had no apparent effect on total activity. The decrease in total activity following the peak at

about 8 to 10 hr indicates that digestion of labeled hay was slowing down and the labeled VFA were being absorbed at a greater rate than they were being produced. It is possible that there was no digestion of labeled hay toward the end of the experiment, and the activity present was merely from labeled VFA that were not yet absorbed. From the level of total activity of the rumen VFA it was concluded that the most rapid breakdown of C^{14} alfalfa hay was during the first 12 hr. Digestion continued at a reduced rate up to 36 hr. There was very little digestion after 36 hr. It should be remembered that in this experiment all of the previous feedings were removed from the rumen and only about a third of the dry matter was present as would be found normally. It is possible that under these conditions the microorganisms attacked the newly ingested hay more rapidly since it was the only available substrate. It is impossible to draw any conclusions concerning cellulose digestion from total activity because some of the activity was probably contributed by proteins and other carbohydrates. It is possible that the peak activity at 8 to 10 hr (Fig. 8) may have been caused by digestion of carbohydrates along with cellulose.

An analysis for C^{14} activity in the rumen dry matter and cellulose at regular intervals following the administration of labeled hay offered a different approach to determining when the labeled hay was digested. It also made available some direct information pertaining to the rate of cellulose digestion. It is apparent from the results presented in Fig. 10 that the digestion of cellulose began shortly after the administration of the labeled hay and proceeded at a rapid rate for the first 24 hr. There was some digestion of cellulose between 24 and 36 hr. At 36 hr the digestion of cellulose appeared to cease. The rate of cellulose digestion in this experiment was in

general agreement with the literature, although there was little evidence of a lag phase.

The termination time of cellulose digestion (Fig. 10) was in general agreement with the termination time exhibited by the total activity (Fig. 8). This suggests that most of the activity in the rumen VFA (Fig. 8) after 12 hr was a result of cellulose digestion. When cellulose digestion stopped there was very little activity in the rumen VFA.

The agreement between Fig. 8 and Fig. 10 (Experiment 4) gave added evidence that total activity was a better method than specific activity for indicating when labeled hay is digested. Between 36 and 48 hr the specific activity (Fig. 7) was approximately one-third of the peak level even though no cellulose was digested during this period. Therefore, specific activity would leave the erroneous impression that digestion was taking place between 36 and 48 hr when actually the breakdown of labeled hay probably terminated at 36 hr when the digestion of cellulose ceased.

It was concluded from these experiments that total activity of VFA was a more reliable measure than specific activity for indicating when labeled hay is digested. While it was necessary to fast the animals in the experiments to study the dilution of specific activity, fasting would not be necessary if one were interested only in total activity of VFA since subsequent feedings have no effect upon total activity. It appears that the rate of degradation of labeled hay could be determined under normal conditions by placing the labeled hay in the normal rumen, feeding unlabeled hay in the usual manner, and determining the total amount of activity in the rumen VFA at regular intervals following administration of the isotope.

While total activity in rumen VFA may be a useful indicator of when

labeled hay is degraded, it does not directly indicate when cellulose is broken down. Since C^{14} activity is present in the non-cellulose portion of a roughage other methods are necessary for determining the rate of cellulose digestion. The technique of determining the total amount of activity in cellulose in the rumen at regular intervals appears to be a useful method. The animal could be maintained under normal conditions and fed in the usual manner. In addition the rate of degradation of sugars, starch, or proteins could be achieved by a modification of this method.

It is recognized that the rate of degradation of alfalfa hay and cellulose in these experiments may be subject to error because the conditions in the rumen were not normal; however, the techniques developed may be of value in future work. It is believed that the continued use of C^{14} labeled alfalfa hay along with improved methods will make a contribution to the present knowledge of rumen metabolism.

CONCLUSIONS

1. Specific activity of rumen VFA was diluted by VFA produced from degradation of previously ingested feed and from degradation of feed introduced into the rumen following the addition of C^{14} labeled alfalfa. This suggests that the level of specific activity will not indicate when C^{14} labeled alfalfa is degraded in the rumen.
2. Polyethylene glycol was a suitable marker for determining the total weight of rumen contents when the rumen contents were removed, strained through cheesecloth and the strained rumen fluid combined with half of the daily hay ration was replaced in the rumen.
3. The level of total activity in the rumen VFA was a more reliable measure

than the level of specific activity of rumen VFA for indicating when C^{14} labeled alfalfa was degraded in the rumen.

4. A technique for determining the rate of cellulose degradation of C^{14} labeled alfalfa was developed. The total activity of cellulose in the rumen at various times was compared with the total activity of cellulose initially introduced into the rumen.

5. The most active period of cellulose degradation in this study occurred during the first 12 hr with little degradation after 36 hr. Abnormal conditions in the rumen may have caused cellulose to be degraded at a more rapid rate than would occur in a normal rumen.

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APPENDIX

Table 1. Production of rumen VFA and its specific activity following feeding of C^{14} labeled alfalfa (Experiment 1)

Hr after administration of isotope	Sample no.[1]	Wt of Na salt of VFA	Micromole of VFA/ml rumen fluid[2]	DPM[3]	DPM/mg of Na salt of rumen VFA
		g			
0.17	1A	0.0037	79.4	564	152
	1B	0.0028	80.2	573	205
0.33	2A	0.0033	76.2	1131	343
	2B	0.0035	77.6	1064	304
0.50	3A	0.0035	74.0	1538	439
	3B	0.0036	76.4	1680	467
1	4A	0.0034	71.8	1880	553
	4B	0.0034	74.0	1971	580
2	5A	0.0039	83.4	10204	2616
	5B	0.0039	35.2	10056	2578
3	6A	0.0042	91.4	10789	2569
	6B	0.0042	92.2	11491	2736
4	7A	0.0045	95.6	20216	4492
	7B	0.0045	97.8	20287	4508
5	8A	0.0040	86.8	12922	3231
	8B	0.0040	90.8	13502	3376
6	9A	0.0044	94.0	11353	2580
	9B	0.0042	98.8	10893	2594
7	10A	0.0038	83.0	8962	2358
	10B	0.0036	81.2	8438	2344
8	11A	0.0038	85.0	6771	1782
	11B	0.0039	88.0	6531	1675
9	12A	0.0035	88.2	5267	1505
	12B	0.0039	86.8	5056	1296
10	13A	0.0026	85.8	3178	1222
	13B	0.0037	88.6	4187	1132
11	14A	0.0037	84.6	3844	1039
	14B	0.0038	85.2	3687	970

Table 1 (cont.)

Hr after administration of isotope	Sample no.[1]	Wt of Na salt of VFA	Micromole of VFA/ml rumen fluid[2]	DPM[3]	DPM/mg of Na salt of rumen VFA
		g			
12	15A	0.0036	83.8	3358	933
	15B	0.0034	81.6	3267	961
14	16A	0.0031	70.0	2629	848
	16B	0.0030	68.6	2473	824
16	17A	0.0031	68.6	2616	844
	17B	0.0031	67.8	2418	780
18	18A	0.0038	60.6	2193	783
	18B	0.0026	56.2	2098	807
20	19A	0.0024	52.0	2042	850
	19B	0.0026	55.2	2096	806
22	20A	0.0023	49.0	1847	803
	20B	0.0024	49.0	1900	792
24	21A	0.0024	49.8	1962	818
	21B	0.0024	48.6	1920	800
27	22A	0.0021	44.0	1280	610
	22B	0.0022	42.8	1360	618
30	23A	0.0019	35.0	1053	554
	23B	0.0019	36.6	1042	548
33	24A	0.0018	33.2	1009	561
	24B	0.0019	33.2	924	486
36	25	0.0037	40.9	1729	467
40	26	0.0025	26.1	1464	586
44	27	0.0020	17.5	1164	582
48	28	0.0021	18.4	1042	496
52	29	0.0027	20.2	1276	469
56	30	0.0023	14.0	1169	508
60	31	0.0026	17.8	971	373

Table 1 (concl.)

Hr after administration of isotope	Sample no.[1]	Wt of Na salt of VFA	Micromole of VFA/ml rumen fluid[2]	DPM[3]	DPM/mg of Na salt of rumen VFA
		g			
64	32	0.0019	11.4	929	489
68	33	0.0017	12.4	1040	612
72	34	0.0013	9.4	729	560

[1] A and B designate duplicates.

[2] VFA titrated with .9225 N NaOH.

[3] Counts per minute corrected for efficiency. See Appendix Table 5.

Table 2. Specific activity of jugular vein blood following feeding of C^{14} labeled alfalfa (Experiment 1)

Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml	Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml
		ml					ml		
2	5A	0.2	0		22	20A	0.2	414	2070
	5B	"	0			20B	"	409	2045
3	6A	"	82	410	24	21A	"	366	1830
	6B	"	30	150		21B	"	263	1315
4	7A	"	52	260	27	22A	"	323	1615
	7B	"	104	520		22B	"	384	1920
5	8A	"	220	1100	30	23A	"	362	1810
	8B	"	319	1595		23B	"	375	1875
6	9A	"	254	1270	33	24A	"	422	2110
	9B	"	444	2200		24B	"	401	2005
7	10A	"	397	1985	36	25A	"	280	1400
	10B	"	509	2545		25B	"	397	1985
8	11A	"	414	2070	40	26A	"	500	2500
	11B	"	496	2480		26B	"	573	2865
9	12A	"	504	2520	44	27A	"	362	1810
	12B	"	401	2005		27B	"	444	2220
10	13A	"	414	2070	48	28A	"	556	2780
	13B	"	466	2330		28B	"	568	2840
11	14A	"	530	2650	52	29A	"	500	2500
	14B	"	478	2390		29B	"	457	2285
12	15A	"	483	2415	56	30A	"	440	2200
	15B	"	435	2175		30B	"	530	2650
14	16A	"	448	2240	60	31A	"	452	2260
	16B	"	397	1985		31B	"	444	2220
16	17A	"	461	2205	64	32A	"	646	3230
	17B	"	504	2520		32B	"	607	3035
18	18A	"	302	1510	68	33A	"	577	2885
	18B	"	332	1660		33B	"	457	2285
20	19A	"	336	1680	72	34A	"	483	2415
	19B	"	289	1445		34B	"	440	2200

[1]Hours after administration of isotope.

[2]A and B designate duplicates.

[3]Counts per minute corrected for efficiency. See Appendix Table 5.

Table 3. Specific activity of saliva following feeding of C^{14} labeled alfalfa (Experiment 1)

Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml	Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml
		ml					ml		
3	6A	0.5	793	1586	24	21A	0.5	298	596
	6B	"	700	1400		21B	"	311	622
4	7A	"	2967	5934	27	22A	"	380	760
	7B	"	2931	5862		22B	"	391	782
5	8A	"	1264	2328	30	23A	"	251	502
	8B	"	1339	2678		23B	"	289	578
6	9A	"	1168	2336	33	24A	"	543	1086
	9B	"	1168	2336		24B	"	747	1494
7	10A	"	915	1830	36	25A	"	251	502
	10B	"	846	1692		25B	"	264	528
8	11A	"	2185	4370	40	26A	"	328	656
	11B	"	2003	4006		26B	"	289	578
10	13A	"	804	1608	44	27A	"	218	436
	13B	"	829	1658		27B	"	176	352
11	14A	"	689	1378	48	28A	"	240	480
	14B	"	623	1246		28B	"	187	374
12	15A	"	879	1758	52	29A	"	220	440
	15B	"	837	1674		29B	"	176	352
14	16A	"	846	1692	56	30A	"	223	446
	16B	"	631	1262		30B	"	149	298
16	17A	"	782	1564	60	31A	"	303	606
	17B	"	785	1570		31B	"	275	550
18	18A	"	675	1350	64	32A	"	99	198
	18B	"	876	1752		32B	"	107	214
20	19A	"	868	1736	68	33A	"	256	512
	19B	"	915	1830		33B	"	287	574
22	20A	"	372	744	72	34A	"	146	292
	20B	"	388	776		34B	"	127	254

[1]Hours after administration of isotope.

[2]A and B designate duplicates.

[3]Counts per minute corrected for efficiency. See Appendix Table 5.

Table 4. Specific activity of urine following feeding of C^{14} labeled alfalfa (Experiment 1)

Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml	Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml
		ml					ml		
0.17	1A	0.5	29	58	12	15A	0.5	13611	27222
	1B	"	51	102		15B	"	13889	27778
0.33	2A	"	687	1374	14	16A	"	11076	22152
	2B	"	687	1374		16B	"	10927	22854
0.50	3A	"	1733	3466	16	17A	"	13622	27244
	3B	"	1716	3432		17B	"	13749	27498
1	4A	"	2404	4808	18	18A	"	10660	21320
	4B	"	2496	4992		18B	"	10798	21596
2	5A	"	14971	29942	20	19A	"	12176	24352
	5B	"	15016	30032		19B	"	12142	24284
3	6A	"	25222	50444	22	20A	"	11571	23142
	6B	"	26064	52128		20B	"	11676	23352
4	7A	"	28011	56022	24	21A	"	10989	21978
	7B	"	27587	55174		21B	"	11144	22288
5	8A	"	24738	49476	27	22A	"	9989	19978
	8B	"	24396	48792		22B	"	9651	19302
6	9A	"	26851	53702	30	23A	"	8240	16480
	9B	"	26776	53552		23B	"	8327	16654
7	10A	"	31071	62142	33	24A	"	7444	14888
	10B	"	30520	61040		24B	"	7487	14974
8	11A	"	30500	61000	36	25A	"	5451	10902
	11B	"	30782	61564		25B	"	5564	11128
9	12A	"	27293	54586	40	26A	"	7018	14036
	12B	"	26542	53084		26B	"	6956	13912
10	13A	"	15124	30248	44	27A	"	8620	17240
	13B	"	14540	29080		27B	"	8444	16888
11	14A	"	11333	22660	48	28A	"	6004	12008
	14B	"	11958	23916		28B	"	5713	11426

Table 4 (concl.)

Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml	Hr[1]	Sample no.[2]	Sample size	DPM[3]	DPM/ml
		ml					ml		
52	29A	0.5	6909	13818	64	32A	0.5	2998	5996
	29B	"	6869	13738		32B	"	2942	5884
56	30A	"	4216	8432	68	33A	"	2513	5026
	30B	"	4102	8204		33B	"	2456	4912
60	31A	"	2311	4622	72	34A	"	2567	5134
	31B	"	2304	4608		34B	"	2456	4912

[1]Hours after administration of isotope.

[2]A and B designate duplicates.

[3]Counts per minute corrected for efficiency. See Appendix Table 5.

Table 5. Determination of efficiency of counter

Type of Sample	Sample no.	Wt standard benzoic C^{14} acid[1]	DPM	CPM	Efficiency	Average efficiency
		g			%	%
Urine	1	0.0117	67392	30306	44.97	
	2	0.0062	35712	16121	45.14	45.06
Saliva	1	0.0104	59904	21866	36.50	
	2	0.0107	61632	22229	36.06	36.28
Blood	1	0.0071	40896	9875	24.15	
	2	0.0059	33984	7853	23.11	
	3	0.0071	40896	9098	22.25	
	4	0.0071	40896	9488	23.20	23.18
Rumen VFA	1	0.0105	60480	27349	45.22	
	2	0.0118	67968	30626	45.06	45.14

[1]Radioactivity 5760 DPM/mg.

Table 6. Production of rumen VFA, specific activity of rumen VFA, and total activity in total rumen VFA following feeding of C^{14} labeled alfalfa (Experiment 4)

Hr after administration of isotope	Sample no.[1]	Wt of Na salt of VFA	Micromole of VFA/ml rumen fluid[2]	DPM[3]	DPM/mg of Na salt of rumen VFA	Microcurie in total rumen VFA
		g				
0.5	2A	0.0125	139.3	3656	292	
	2B	0.0123	149.7	3113	253	
1	3A	0.0112	136.0	4956	443	
	3B	0.0109	122.6	4673	429	
2	4A	0.0076	84.8	10051	1323	148
	4B	0.0079	98.0	9633	1219	
3	5A	0.0086	94.7	16844	1959	
	5B	0.0086	98.0	17424	2026	
4	6A	0.0093	106.2	20800	2237	278
	6B	0.0100	127.7	20884	2088	
5	7A	0.0109	114.5	22507	2065	
	7B	0.0122	135.4	23327	1912	
6	8A	0.0099	114.5	22573	2280	
	8B	0.0098	116.7	24216	2471	
7	9A	0.0102	114.5	22262	2183	
	9B	0.0091	104.6	22056	2424	
8	10A	0.0085	98.0	22153	2606	278
	10B	0.0082	99.1	21860	2666	
9	11A	0.0090	100.2	22036	2448	
	11B	0.0081	95.8	21667	2675	
10	12A	0.0078	89.2	20780	2664	
	12B	0.0080	91.4	21242	2655	
11	13A	0.0084	100.2	20898	2488	
	13B	0.0081	93.0	21609	2668	
12	14A	0.0074	82.0	17900	2419	228
	14B	0.0080	92.5	17887	2236	
16	15A	0.0067	76.0	14482	2160	199
	15B	0.0062	70.5	14536	2345	

Table 6 (cont.)

Hr after administration of isotope	Sample no.[1]	Wt of Na salt of VFA	Micromole of VFA/ml rumen fluid[2]	DPM[3]	DPM/mg of Na salt of rumen VFA	Microcurie in total rumen VFA
		g				
20	16A	0.0057	63.3	11729	2058	
	16B	0.0055	65.5	10980	1996	
24	17A	0.0050	58.9	9227	1845	89
28	18A	0.0040	46.2	5764	1441	54
	18B	0.0037	42.4	5840	1578	
32	19A	0.0035	38.5	4833	1381	46
	19B	0.0034	37.4	4726	1390	
36	20A	0.0027	20.8	2989	1107	32
	20B	0.0029	35.8	2816	971	
40	21A	0.0028	32.5	2649	946	30
	21B	0.0025	27.5	2760	1104	
44	22A	0.0028	33.6	2226	795	23
	22B	0.0024	28.1	2256	940	
48	23A	0.0026	27.5	1933	743	20
	23B	0.0025	25.9	1969	788	
48.5	24A	0.0037	42.9	1647	445	
	24B	0.0039	47.3	1731	444	
49	25A	0.0049	55.6	1458	298	
	25B	0.0042	46.8	1400	333	
50	26A	0.0059	65.0	1391	236	16
	26B	0.0062	66.6	1333	215	
51	27A	0.0067	69.4	1249	186	
	27B	0.0074	79.3	1218	165	
52	28A	0.0073	89.7	942	129	16
	28B	0.0071	90.3	958	135	
53	29A	0.0065	83.7	889	137	
	29B	0.0069	77.6	938	136	
54	30A	0.0068	87.0	747	110	
	30B	0.0069	90.3	727	105	

Table 6 (concl.)

Hr after administration of isotope	Sample no.[1]	Wt of Na salt of VFA	Micromole of VFA/ml rumen fluid[2]	DPM[3]	DPM/mg of Na salt of rumen VFA	Microcurie in total rumen VFA
		g				
55	31A	0.0057	67.7	587	103	
	31B	0.0058	71.6	553	95	
56	32A	0.0060	76.0	636	106	11
	32B	0.0068	78.7	658	97	
57	33A	0.0053	72.7	447	84	
	33B	0.0046	68.3	456	99	
58	34A	0.0058	72.7	478	82	
	34B	0.0059	77.1	473	80	
59	35A	0.0042	54.0	373	89	
	35B	0.0043	65.0	300	70	
60	36A	0.0049	63.9	416	85	6
	36B	0.0051	73.8	356	70	

[1] A and B designate duplicates.

[2] VFA titrated with .1101 N NaOH.

[3] Counts per minute corrected for efficiency. See Appendix Table 5.

Table 7. Specific activity of dry matter or cellulose in rumen contents and total activity in dry matter or cellulose at various times following the feeding of C^{14} alfalfa (Experiment 4)

Hr after administration of isotope	Specific activity microcuries/g		Total microcuries in rumen		% Cellulose removed
	Dry matter	Cellulose	Dry matter	Cellulose	
2	1.218	0.997	4431	1089	20.5
4	1.182	1.000	3891	1070	21.9
8	1.173	1.026	2901	780	43.1
12	1.181	0.698	2840	501	63.4
16	1.065	0.671	2624	449	67.2
24	0.842	0.673	1431	289	78.9
28	0.607	0.605	945	237	82.7
32	0.601	0.431	977	175	87.2
36	0.580	0.425	1086	232	83.1
40	0.300	1.014	609	615	55.1
44	0.263	0.377	543	238	82.6
48	0.226	0.413	491	278	79.7
50	0.174	0.148	516	139	89.9
52	0.186	0.102	769	134	91.2
56	0.197	0.211	730	244	82.2
60	0.136	0.163	444	172	87.4

TECHNIQUES FOR DETERMINING THE RATE OF METABOLISM OF C^{14} LABELED ALFALFA IN THE BOVINE RUMEN

by

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It has been difficult to determine when cellulose is degraded in the rumen because cellulose from any given feeding loses its identity when it is mixed with cellulose that remains from previously ingested feed. The use of C^{14} labeled alfalfa provides a means of identifying the cellulose from a given feeding and therefore, may provide a method for determining when cellulose is degraded in the rumen.

An animal was fasted for 72 hr following the initial addition of C^{14} alfalfa and unlabeled alfalfa. Specific activity of rumen VFA increased rapidly, peaked at 4 hr and then decreased rapidly until the twelfth hr. Thereafter, specific activity remained at a low level until the experiment was terminated. It was postulated that the rapid decrease in specific activity was due to dilution by VFA resulting from previously ingested feed. To test this an experiment was conducted in which the rumen was emptied and refilled with strained rumen fluid, C^{14} labeled alfalfa, and a regular feeding of unlabeled alfalfa. The animal was fasted for 48 hr. Specific activity increased rapidly, peaked at 9 hr, and then gradually declined to 48 hr. The slower rate of decline in specific activity as compared with the first experiment suggests that VFA from previous feedings dilute specific activity. When additional unlabeled alfalfa was placed in the rumen at 48 hr, specific activity dropped precipitously, thus indicating that subsequent feedings also dilute specific activity.

Total activity of rumen VFA was calculated by determining the weight of rumen contents with polyethylene glycol and determining the amount of activity in a known weight of rumen contents. C^{14} labeled alfalfa was placed in an empty rumen with unlabeled alfalfa and strained rumen fluid and the animal was then fasted for 48 hr. The maximum total activity in the rumen VFA

occurred at 4 to 8 hr and then declined. There was little activity remaining after 36 hr.

While total activity appears to be a satisfactory indicator of the rate of degradation of alfalfa, it does not specifically indicate when cellulose is degraded because other components of the hay are labeled. The total amount of cellulose and the specific activity of cellulose in the rumen was determined at regular intervals following the addition of C^{14} labeled alfalfa, unlabeled alfalfa, and strained rumen fluid to an empty rumen. The total activity of cellulose in the rumen at various times was compared with the total activity of cellulose initially introduced into the rumen. The most active degradation of cellulose occurred during the first 12 hr and there was little degradation after 36 hr.

Although the conditions in this study were abnormal because the undigested feed residue in the rumen was removed and the animal was fasted, it would appear that the techniques developed to determine the rate of cellulose breakdown might be used when an animal is fed in a normal manner.