Formation of Short-Chain Fatty Acids from Cellulose, Starch, and Metabolic Intermediates by Ovine and Bovine Rumen Microorganisms

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Incubation of ovine rumen microorganisms in vitro with starch, maltose, or glucose, in combination with cellulose increased the amount and presumably the rate of formation of acetic, propionic, and butyric acids. Digestion of cellulose by bovine rumen microorganisms in vitro was decreased by alanine, α-ketoglutarate, and lactate, whereas the other metabolic acids added increased the formation of short-chain fatty acids. Approximately 40% of the carbon in cellulose and 50% of the carbon of glucose was recovered in the short-chain fatty acids. Further evidence is presented to support the hypothesis that the major pathway of propionic acid formation in rumen bacteria involves succinate decarboxylation.

ACETIC, PROPIONIC, AND BUTYRIC ACIDS are the major end products formed during the fermentation of carbohydrates by rumen microorganisms (*9*, *17*). Marston (*17*), Schambye and Phillipson (*18*), and Carroll and Hungate (*6*) have estimated that these short-chain fatty acids provide from 40 to 100% of the resting energy requirements of ruminant animals.

Carroll and Hungate (*6*) investigated the rate of volatile fatty acid production by steers on grain, hay, and pasture by incubating whole rumen contents in vitro, and found that the rates were 55, 36, and 23 equivalents of volatile fatty acid per 100 kg. of rumen contents per day, respectively.

Although the rate of fermentation on the grain ration was most rapid, little is known of the percentage conversion of carbohydrates to fatty acids by rumen microorganisms during a 24-hour fermentation period.

The following study was made to determine the fatty acids formed from cellulose, starch, maltose, glucose, pyruvate, lactate, and combinations of these substances with cellulose; the percentage conversion of cellulose and glucose to fatty acids; the effect of low concentrations of metabolic intermediates on the activity of the cellulolytic microflora; and the percentage conversion of metabolic intermediates to fatty acids by rumen microorganisms in vitro.

Experimental

Studies with Ovine Rumen Microorganisms. Ovine rumen microorganisms were obtained from two wether lambs at the Ohio State University, fed alfalfa hay and a limited amount of concentrate composed of corn and oats. The rumen contents were removed via a permanent rumen fistula, and the rumen fluid, containing the microorganisms, was separated from the plant material by squeezing the contents through two layers of cheesecloth. One hundred milliliters of the whole rumen fluid was used as the inoculum for a 450-ml. fermentation medium (*14*). The composition of the basal medium is given in Table I. The pH was adjusted to 6.6 to 6.8 every few hours with 2*M* sodium carbonate. After a 24-hour fermentation period, the volume was made up to 450 ml. and samples were withdrawn for cellulose, protein, and volatile fatty acid analyses. Cellulose was estimated by the method of Crampton and Maynard (*7*), protein by the Kjeldahl determination of the nitrogen insoluble in 10% (w./v.) trichloroacetic acid and volatile fatty acids by the method of Harper (*12*).

Cellulose (Solka-Floc 40 A) was used as the reference carbohydrate and was added at the 1% level. Cornstarch, maltose, glucose, sodium pyruvate, and lactic acid (85%) were each added separately and in combination with cellulose at levels which were equivalent to the carbon content of 4.5 grams of cellulose, in order to observe the effect of each on the growth of the total population of rumen microorganisms, cellulytic activity of the cellulose digesting rumen microorganisms and production of acetic, propionic, and butyric acids.

Table I. Composition of Basal Medium

Constituent	Grams per 100 Ml.
Cellulose	1.00
Urea	0.168
Na_2CO_3	0.150
Na_2HPO_4	0.113
NaH_2PO_4	0.109
NaCl	0.043
KCl	0.043
Na_2SO_4	0.015
$MgCO_3$	0.004
$CaCl_2$	0.004
$FeCl_3.6H_2O$	0.0044

Studies with Bovine Rumen Microorganisms. Bovine rumen microorganisms were obtained from an alfalfa hay–fed steer fitted with a permanent rumen fistula, using described methods (*4*). The bacteria were separated from the rumen liquor with a Sharples supercentrifuge. The sediment from 40.0 ml. of rumen liquor, suspended in 8 ml. of 0.005*M* phosphate buffer pH 7.0, plus 10 ml. of supernatant from the Sharples centrifugation, constituted the inoculum for the 100-ml. in vitro fermentation medium which is described in Table I. Unless otherwise indicated, the fermentation time was 30 hours. Carbon dioxide was bubbled through the flasks to establish anaerobic conditions.

Two millimoles of the compounds to be tested were added per 100 ml. of medium, with the exception of glucose, which was added at either the 0.55-, 0.62-, 1.0-, or 1.62-mmole levels.

Acetic, propionic, butyric, lactic, α-ketoglutaric, and malonic acids were determined by chromatographic separation on a silica gel column by combining the procedures of Elsden (*9*) and Bulen, Varner, and Burrell (*5*). Satisfactory recoveries of 95 to 100% were obtained by this procedure. This method did not separate butyric acid from the higher

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acids, valeric and isovaleric, which have been shown to be present in the rumen in small quantities (*1, 11*).

Following the fermentation period, cellulose was determined by the method of Crampton and Maynard (*7*).

Results and Discussion

Ovine Rumen Microorganisms. The effect of starch, maltose, glucose, lactate, and pyruvate, added at the 25% carbon level, and starch, pyruvate, and lactate at the 50% carbon level, on the rate of cellulose digestion is given in Table II. With the exception of glucose, all of the substances added at the 25% level increased the rate of cellulose digestion. However, at the 50% level, lactate completely inhibited cellulose digestion while the inhibitory effect of starch and pyruvate was less pronounced. This stimulation and depression of the rate of cellulose digestion with low and high levels of soluble carbohydrates, respectively, are in agreement with the results of Arias and coworkers (*2*) with bovine rumen microorganisms.

Table II also shows that the amount, and presumably the rate of formation, of volatile fatty acids formed from starch, maltose, glucose, lactate, and pyruvate was greater than from cellulose alone. When lactate or pyruvate supplied 50% or more of the carbon as in Experiment B, Table II, the preferential formation of acetate occurred with pyruvate, whereas greater propionate formation relative to acetate occurs with lactate. Wherever part of the cellulose was replaced with a soluble carbon source, butyric acid formation was increased. This may be of considerable importance in high energy rations for ruminant animals.

Because somewhat variable results were obtained with ovine rumen microorganisms, further studies were made with bovine rumen microorganisms to extend these preliminary results.

Bovine Rumen Microorganisms. The experiments with bovine rumen microorganisms were designed to determine the effect of low concentrations (1 to 2 mmoles) of glucose and metabolic acids on the cellulolytic activity of rumen microorganisms, the percentage conversion of cellulose, glucose, and metabolic acids to volatile fatty acids, the relative proportions of fatty acids formed from the various carbon sources, and, if possible, a general elucidation of metabolic pathways relative to the formation of volatile fatty acids.

The results in Table III show that a concentration as low as 2 mmoles of lactate, alanine, or α-ketoglutarate per 100 ml. depressed the rate of cellulose digestion, whereas glucose, pyruvate, succinate, aspartate, malate, glutamate, or malonate at the same concentration had little or no effect on the rate of cellulose digestion.

With the exception of alanine, all the carbon sources added to the basal medium containing 1 gram of cellulose increased the formation of either acetic, propionic, or butyric acids above that expected from the digested cellulose (Table III). The increased formation of these fatty acids may have been due to the actual conversion of the added carbon source to the fatty acids, or its influencing a more efficient conversion of the digested cellulose to fatty acids. Assuming the former to be the major factor, the per cent carbon recovered as fatty acids was calculated as per cent of the carbon source added, taking into consideration the fatty acids contributed by the inoculum and the per cent cellulose digested.

In the basal flask which contained 1 gram of cellulose and 0.55 mmoles glucose, 32.4% of the carbon in the digested cellulose and glucose was accounted for in the fatty acids formed.

The additional carbon sources characteristically increased the formation of either acetic, propionic, and butyric acids, both acetic and propionic acid, only acetic acid, or only propionic acid.

Glucose, pyruvate, and lactate increased the formation of butyric acid whereas all the other carbon sources had no effect on butyric acid formation. Glucose, pyruvate, lactate, and malate increased both acetic and propionic acid formation. Malonate, glutamate, and α-ketoglutarate increased the formation of acetic acid. Thus, it appears that related metabolic intermediates react differently and specifically when added to a growing, mixed culture of rumen microorganisms.

A further study was made to determine the rate of fermentation and the relative rates of formation of acetic, propionic, and butyric acids from cellulose, glucose, pyruvate, lactate, and alanine in flasks containing 1% cellulose. The results are given in Table IV expressed as per

Table III. Conversion of Cellulose, Glucose, and Metabolic Intermediates to Volatile Fatty Acids by Bovine Rumen Microorganisms[a]

Carbon Sources	Carbon Recovered, %				Cellulose Digested, %
	Acetic	Propionic	Butyric	Total	
Basal[b]	14.1	17.0	1.3	32.4	60.3
Basal + glucose[c]	17.9	20.5	9.4	47.8	54.6
Basal + pyruvate	48.3	23.0	8.3	79.6	72.9
Basal + lactate	44.1	33.9	15.5	93.5	31.9
Basal + alanine	−1.3	−14.1	0.7	−14.7	21.6
Basal + succinate	−13.1	27.8	−1.1	13.6	53.4
Basal + aspartate	−2.2	25.9	1.0	24.7	63.7
Basal + malate	15.6	19.5	−1.4	33.7	58.0
Basal + α-ketoglutarate	23.8	0.5	1.6	25.9[d]	21.6
Basal + glutamate	20.7	9.6	3.3	33.6	63.7
Basal + malonate	55.4	8.7	−0.7	63.4	62.5

[a] Recovery estimated as per cent of added carbon recovered from 30-hour fermentation based on carbon in substrate.
[b] One gram cellulose plus 0.55 mmole glucose per 100 ml. medium.
[c] One mmole glucose per 100 ml. Two mmoles of the other carbon substrates were added.
[d] 18.1% α-ketoglutarate remained in flask.

Table II. Conversion of Cellulose, Starch, Maltose, Glucose, Pyruvate and Lactate to Volatile Fatty Acids by Ovine Rumen Microorganisms in Vitro

Carbon Source[a]	Millimoles Acid per 100 Ml.				Cellulose Digested, %
	Acetic	Propionic	Butyric	Total	
Experiment A					
Cellulose	0.88	0.56	0.17	1.61	13.4
Cellulose:starch (3:1)	1.42	1.88	0.79	4.09	31.1
Cellulose:maltose (3:1)	1.61	1.86	0.59	4.06	30.5
Cellulose:glucose (3:1)	1.81	1.89	0.56	4.26	18.3
Cellulose:pyruvate (3:1)	2.46	1.73	0.46	4.65	32.0
Cellulose:lactate (3:1)	1.71	1.79	0.47	3.97	38.0
Experiment B					
Cellulose	2.81	1.90	0.59	5.30	35.5
Cellulose:starch (1:1)	3.06	2.45	1.35	6.86	29.2
Starch	2.95	2.82	1.77	7.54	..
Maltose	3.99	1.90	1.64	7.53	..
Glucose	4.29	2.98	1.51	8.78	..
Cellulose:pyruvate (1:1)	4.79	1.67	1.04	7.50	32.7
Pyruvate	6.76	0.90	1.08	8.74	..
Cellulose:lactate (1:1)	2.91	3.03	1.08	7.02	0.5
Lactate	4.15	4.51	0.32	8.98	..

[a] Total carbon in each flask was equivalent to carbon in 1.0 gram cellulose per 100 ml. of fermentation medium.

cent substrate carbon recovered in the various fatty acids. The data for acetate and propionate production over a 48-hour fermentation period are presented in Figures 1 and 2, expressed as millimoles of acids formed.

The initial rate of fatty acid formation, with cellulose as the only carbon substrate, was rather low, acetic acid being the first acid to be formed in quantity (Figure 1). After 15 hours, propionic acid began to be formed rapidly and reached about the same concentration as acetic acid after 48 hours of fermentation (Figure 2). With the exception of alanine, all the added carbon sources increased the initial rate of formation of fatty acids. Glucose and lactate served to increase the rate of formation of both acetic and propionic acids, whereas pyruvate only increased the rate of formation of acetic acid. Conversely, alanine preferentially decreased the rate of formation of propionic acid.

The interpretation of these results is more significant if the following over-all reactions postulated by several English workers (*10*, *15*) are considered:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow 2\,\underset{\text{pyruvic}}{C_3H_4O_3} + 4H^+ + 4E \quad (1)$$

$$\underset{\text{pyruvic}}{C_3H_4O_3} + H_2O \rightarrow \underset{\text{acetic}}{C_2H_4O_2} + 2H^+ + 2E + CO_2 \quad (2)$$

$$\underset{\text{pyruvic}}{C_3H_4O_3} + 2H^+ + 2E \longrightarrow \underset{\text{lactic}}{C_3H_6O_3} \quad (3)$$

$$\underset{\text{lactic}}{C_3H_6O_3} + 2H^+ + 2E \rightarrow \underset{\text{propionic}}{C_3H_6O_2} + H_2O \quad (4)$$

Over-all reaction of glucose to fatty acids:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{acetic}}{C_2H_4O_2} + \underset{\text{propionic}}{C_3H_6O_2} + 2H^+ + CO_2 \quad (5)$$

The data in Table III provide further evidence that glucose is a major intermediate in the digestion of cellulose, for the relative amounts of acetic and propionic acids formed from glucose were nearly the same as from cellulose. In a study of bacterial cellulases, Hungate (*13*) found that cellobiose was the sole product of cellulose breakdown by autolyzing cultures of *Clostridium cellobioparus*. Kitts and Underkofler (*16*) recently reported that glucose was the major end product of cellulase activity of rumen organisms, although a trace of cellobiose was also found.

The same general results were obtained with added lactate as with glucose although lactate appeared to depress the rate of cellulose digestion. Added pyruvate was dissimilated primarily to acetic acid by Reaction 2. Although it might appear logical that, during glucose metabolism, lactic acid should be formed at the same rate as acetic acid, as the results for the first 5 hours of fermentation in Table V suggest, Reactions 3 and 4 require two additional reduced diphosphopyridine nucleotide (DPN) molecules for the lactic acid to be converted to propionic acid. This may be accomplished by an additional dismutation of a mole of pyruvic acid in which case 2 moles of acetate would be formed per mole of propionic. The results in Table III would substantiate the above hypothesis.

Table V. Formation of Acetic and Lactic Acids from Glucose and Pyruvate in 5 Hours

Carbon Source	Millimoles Acid Formed Per 100 Ml. Acetic	Lactic
Cellulose + glucose[a]	0.29	0.28
Cellulose + glucose + glucose[b]	0.50	0.72
Cellulose + glucose + pyruvate[c]	0.65	0.45
Cellulose + glucose + alanine[c]	0.34	0.22

[a] 0.62 mmole per 100 ml.
[b] 1.0 mmole per 100 ml.
[c] 2.0 mmoles per 100 ml.

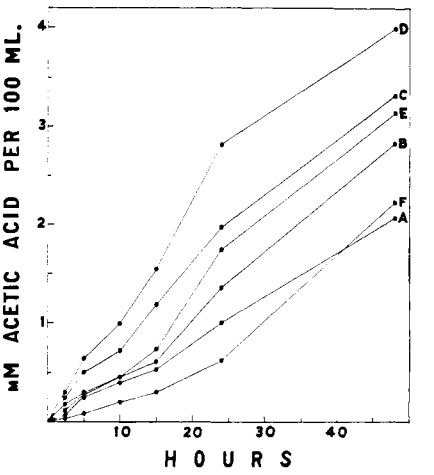

Figure 1. Effect of fermentation time on formation of acetic acid from cellulose or cellulose plus various substrates by bovine rumen microorganisms in vitro

A. Cellulose
B. A + 0.62 mmole of glucose
C. B + 1.0 mmole of glucose
D. B + 2.0 mmoles of pyruvate
E. B + 2.0 mmoles of lactate
F. B + 2.0 mmoles of alanine

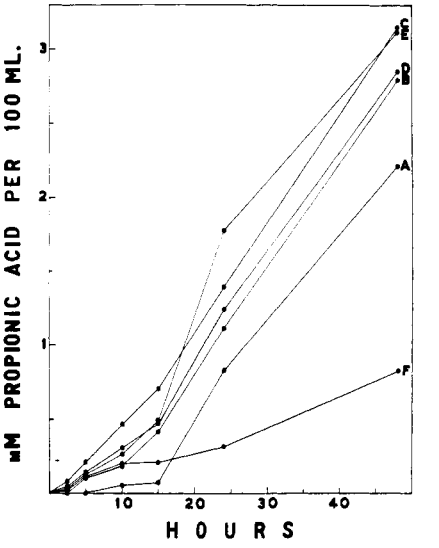

Figure 2. Effect of fermentation time on formation of propionic acid from cellulose or cellulose plus various substrates by bovine rumen microorganisms in vitro

A. Cellulose
B. A + 0.62 mmole of glucose
C. B + 1.0 mmole of glucose
D. B + 2.0 mmoles of pyruvate
E. B + 2.0 mmoles of lactate
F. B + 2.0 mmoles of alanine

Table IV. Conversion of Cellulose, Glucose, Pyruvate, Lactate, and Alanine to Fatty Acids by Bovine Rumen Microorganisms[a]

Carbon Source	Carbon Recovered, % Acetic	Propionic	Butyric	Total	Cellulose Digested, %
Cellulose	15.9	23.8	1.4	41.1	75.7
Cellulose + glucose[b]	19.8	25.9	10.7	56.4	84.0
Cellulose + lactate[c]	16.3	42.5	9.6	68.4	74.0
Cellulose + glucose[b]	16.3	24.0	2.4	42.7	84.0
Cellulose + glucose + glucose[d]	18.9	15.1	13.2	47.2	86.5
Cellulose + glucose + pyruvate[c]	38.7	3.2	5.2	47.1	84.6
Cellulose + glucose + lactate[c]	13.2	15.6	17.7	46.5	84.4
Cellulose + glucose + alanine[c]	17.8	−34.0	12.6	−3.6	41.3

[a] Recovery estimated as per cent of added carbon recovered from 48-hour fermentation based on carbon in the underlined substrate.
[b] 1.62 mmoles per 100 ml.
[c] 2.0 mmoles per 100 ml.
[d] 1.0 mmole per 100 ml.

The effect of lactate in decreasing the rate of cellulose digestion may partially be explained by its being an intermediate in the conversion of pyruvate to propionic acid. For the reduction of lactate to propionate to occur, two reduced DPN molecules are required. These may theoretically be obtained by the oxidation of lactate to pyruvate, but the accumulation of pyruvate depresses the rate of glycolysis (Reaction 1). Although pyruvate was not detected in the

reaction medium, the presence of a relatively high concentration of lactate may interfere with the pyruvate dismutation reaction.

The inhibition of cellulose digestion by alanine cannot readily be explained. The rates of formation of acetic and lactic acids in the early stages of the fermentation were not affected, although propionic acid formation was definitely decreased after 10 hours of fermentation.

Succinate has previously been shown to be quantitatively decarboxylated to propionic acid by resting rumen bacteria (*8*, *10*, *15*, *19*) and aspartate has been shown by Sirotnak, and coworkers (*20*) to be reductively deaminated to succinate.

The low recovery (less than 25%) of succinate and aspartate carbon in fatty acids may have been due to their incorporation into cellular constituents, because no succinate remained in the flask at the end of the 30-hour fermentation period. Furthermore, the low recovery cannot be explained on the basis of experimental error for the recovery of malonate carbon as acetic acid approached theoretically expected recovery (85%).

Glutamic acid and α-ketoglutarate did not appear to be metabolized via the citric acid cycle for practically no propionic acid was formed from these substrates. However, the fact that between 20 and 25% of the carbon from both substrates was recovered in acetic acid would suggest that they were both metabolized in a similar manner. The effect of α-ketoglutarate in decreasing the rate of cellulose digestion cannot be explained on the basis of the citric acid cycle, for succinate and malate exhibited on such effect. However, further observations in this laboratory indicate that 2-mmole concentrations of oxalacetate and citrate also decrease the rate of cellulose digestion. The interpretation of these results is not apparent at the present time.

Within the past 15 years, some evidence has accumulated on the mechanism of propionate formation by anaerobic bacteria. Pyruvate may be reduced either through lactate and acrylate, or it may be converted to oxalacetate by carbon dioxide fixation, reduced to succinate, and decarboxylated to propionate.

Barker and Lipmann (*3*) found that lactate utilization in propionic acid bacteria could be inhibited by sodium fluoride at a concentration which did not inhibit pyruvate utilization. Wood and coworkers (*21*, *22*) found that carbon-13 was incorporated into the carboxyl group of propionate by *Propionibacterium* in the presence of carbon-13–labeled carbon dioxide and suggested that propionic acid might arise via fixation of carbon dioxide by pyruvate followed by decarboxylation of a symmetrical dicarboxylic acid. Delwiche (*8*) observed succinic decarboxylase activity with washed cells of *Propionibacterium pentosaceum* and postulated a mechanism of propionic acid formation through carbon dioxide fixation and decarboxylation according to the following reactions:

$$\text{Lactate} \xrightarrow{+CO_2} \text{malate} \xrightarrow{-H_2O} \text{fumarate} \xrightarrow{+2H} \text{succinate} \xrightarrow{-CO_2} \text{propionate}$$

Johns (*15*) isolated a micrococcus from the rumen of sheep which quantitatively converted succinate to propionate and carbon dioxide. This decarboxylation of succinate was confirmed by Sijpesteijn and Elsden (*19*) and Sirotnak and coworkers (*20*) using washed rumen microorganisms.

Furthermore, Delwiche (*8*) observed that succinic decarboxylase activity as well as propionate formation from pyruvate with washed cells of *P. pentosaceum* was almost completely inhibited by 0.3*M* malonate. The results in Table VI lend further support to the hypothesis that propionic acid formation in rumen bacteria occurs primarily through succinate decarboxylation, for the formation of propionate from added pyruvate and lactate was significantly reduced in the presence of 2 mmoles of malonate.

It is of interest to use the rate of fatty acid production as reported in Table IV to predict the amount of fatty acids produced under in vivo conditions. Such calculations can only be based on the assumption that the in vitro measurements of rate approximate those occurring under natural conditions. In the flask containing 1 gram of pure wood cellulose substrate, 4.54 mmoles of total acid was formed in 48 hours or 2.27 mmoles in 24 hours based on the following calculations:

1 gram of cellulose × 75.7% cellulose digested × mg. of carbon per gram of cellulose (444) × % of carbon recovered as fatty acid ÷ % carbon in fatty acid × equivalent weight of acid = millimoles of acid formed per 48 hours

The sum of such calculations for each acid would give the total. In the flask to which 1.62 mmoles of glucose was added, the total fatty acid production was 3.52 mmoles (Table IV) using the same method of calculation.

If these data are applied to a steer consuming 10 kg. of cellulose equivalent (equal to about 30 kg. of hay) where the predominant carbon source was cellulose, the calculated fatty acid production would be 23 moles per day. If the ration contained the equivalent of roughly 2 parts of cellulose plus 1 part of glucose (1 gram of cellulose + 1.62 millimoles of glucose as in Table IV), then the calculated acid production from the 10 kg. of cellulose-soluble carbohydrate equivalent would be 35 moles per day. The rate and amount of fatty acid in vivo production are difficult to ascertain, but Carroll and Hungate (*6*) estimated a production of 23 and 36 moles of fatty acids per 100 kg. of rumen contents per day for steers on pasture and hay, respectively, when whole rumen contents were used. Ten kilograms of feed equivalent per day and 100 kg. of rumen contents are roughly equivalent; thus, these two methods for calculating the total acid production gave approximately the same results. This similarity would lend support to the validity of the in vitro system used in these studies.

Summary

Starch, maltose, glucose, pyruvate, and lactate were incubated with ovine rumen microorganisms in vitro as the sole carbon source and in combination with cellulose. The results indicated that combinations of starch, maltose, or glucose with cellulose increased the amount and presumably the rate of formation of acetic, propionic, and butyric acids. Pyruvate and lactate preferentially increased the rate of formation of acetic and propionic acid, respectively.

Pyruvate, lactate, alanine, malate, succinate, aspartate, malonate, α-ketoglutarate, and glutamate were incubated with cellulose digesting, bovine rumen

Table VI. Effect of Malonate on Conversion of Pyruvate and Lactate to Volatile Fatty Acids[a]

Carbon Source	Carbon Recovered, %				Cellulose Digested, %
	Acetic	Propionic	Butyric	Total	
Basal[b]	14.1	17.0	1.3	32.4	60.3
Basal + malonate	55.4	8.7	−0.7	63.4	62.5
Basal + pyruvate	48.3	23.0	8.3	79.6	72.9
Basal + malonate + pyruvate	32.7	7.5	2.4	42.6	58.0
Basal + lactate	44.1	33.9	15.5	93.5	31.9
Basal + malonate + lactate	5.4	−4.3	21.0	22.2	26.2

[a] Carbon recovered from 30-hour fermentation based on the carbon in the underlined substrate.

[b] One gram cellulose plus 0.55 mmole glucose per 100 ml. medium. Two millimoles of malonate, pyruvate, and lactate were added.

microorganisms in vitro at the 0.002*M* level to determine their effect on the rate of cellulose digestion and short-chain fatty acid formation.

Alanine, α-ketoglutarate, and lactate decreased the rate of cellulose digestion, whereas the other metabolic acids that were added had no effect on the rate of cellulose digestion.

Approximately 40% of the carbon in cellulose was recovered in the short-chain fatty acids, acetic, propionic, and butyric acid, whereas approximately 50% of the carbon of glucose was recovered in these fatty acids.

All the metabolic acids except alanine that were added to the fermentation medium were metabolized to some extent to short-chain fatty acids. Added pyruvate, malonate, α-ketoglutarate, and glutamate primarily increased the formation of acetic acid. Succinate and aspartate primarily increased the formation of propionic acid, whereas lactate and malate increased the formation of both acetic and propionic acid in approximately the same proportion.

The added metabolic intermediates affect major metabolic pathways involved in the metabolism of glucose to volatile fatty acids. The major pathway of propionic acid formation in rumen bacteria involves succinate decarboxylation.

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FOOD STERILIZATION

Stability of Certain B Vitamins Exposed to Ethylene Oxide in the Presence of Choline Chloride

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Exposure to ethylene oxide of crystalline B vitamins suspended in starch with choline chloride added resulted in the destruction of practically all the thiamine and large amounts of the riboflavin, pyridoxine, niacin, and folic acid. The effect of the ethylene oxide on thiamine may be due in part to the increase in pH that occurred in the presence of choline chloride. The alkalinity may explain the destruction of the thiamine but cannot explain the destruction of niacin, riboflavin, or folic acid. The mechanisms for the latter reactions are unknown. Cocarboxylase was also destroyed under the above conditions. About 40% of the thiamine in a stock diet was destroyed following exposure to ethylene oxide. There was no destruction of pantothenic acid, biotin, or vitamin B_{12} when the vitamin mixtures were exposed to ethylene oxide.

Ethylene oxide has been used for the chemical sterilization of a number of substances including foods. Although only a few foods are currently being treated with this compound, there are suggestions that the method might be valuable for many other foods (*3*).

The current interest in ethylene oxide prompted an extension of the previous observation which indicated on the basis of rat growth studies that thiamine in purified diets was destroyed on exposure to the gas (*7*). The poor growth of the rats on the diet treated with ethylene oxide could be partially overcome by feeding extra supplements of thiamine. Furthermore, a small percentage of the rats fed the treated diet showed neuromuscular changes indicative of a thiamine deficiency.

In order to evaluate the influence of ethylene oxide upon the B vitamins in the purified diet, starch-vitamin mixtures were exposed to the gas. The individual vitamins, as well as a combination of the individual vitamins, were dispersed in a choline chloride–starch mixture and exposed to ethylene oxide. Choline chloride was included in each mixture because earlier work (*7*) showed that this compound played an important role in the destruction of thiamine whereas choline citrate appeared to be much less active.

Experimental

The vitamin mixture which was used in the purified diet at a level of 2%