

THE ISOLATION AND FERMENTATION CHARACTERISTICS OF
BUTYRIVIBRIO SPECIES FROM RUMINAL INGESTA

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LITERATURE REVIEW

BUTYRIVIBRIO IS A NEW GENUS PROPOSED BY BRYANT AND SMALL (1956A) FOR THOSE ANAEROBIC, NONSPOREFORMING, MONOTRICHOUS, GRAM NEGATIVE, CURVED RODS WHICH FERMENT GLUCOSE WITH THE PRODUCTION OF LARGE AMOUNTS OF BUTYRIC ACID. SEVERAL OTHER WORKERS HAVE ALSO ENCOUNTERED ANAEROBIC, GRAM NEGATIVE, BUTYRIC ACID-PRODUCING CURVED RODS IN THE RUMEN. HUNGATE (1950) REPORTED A "LESS ACTIVELY CELLULOLYTIC ROD" WHICH PRODUCED LARGE AMOUNTS OF BUTYRIC AND SOME LACTIC AND FORMIC ACIDS, AND IS UNDOUBTEDLY A MEMBER OF THE GENUS. THE RO-H TYPE OF CURVED RODS DESCRIBED BY HUHTANEN AND GALL (1952) ARE PROBABLY MEMBERS OF THIS GENUS, ALTHOUGH THESE ORGANISMS ARE RATHER SMALL COMPARED TO THE CURVED RODS ISOLATED BY BRYANT AND SMALL (1956A), AND THEY FREQUENTLY SHOW DEEPLY STAINED SWELLINGS IN OLD CULTURES. WILSON (1953) REPORTED ANAEROBIC, SMALL SLENDER, MONOTRICHOUS RODS THAT PRODUCED BUTYRIC, ACETIC AND LACTIC ACIDS FROM GLUCOSE. THESE ORGANISMS WERE BELIEVED TO BE A STABLE PART OF THE RUMEN FLORA OF SHEEP AND CERTAINLY BELONG TO THE GENUS BUTYRIVIBRIO. THE SUBGROUP B-1 OF GRAM NEGATIVE, CURVED RODS PRODUCING A LARGE AMOUNT OF BUTYRIC ACID FROM GLUCOSE DESCRIBED BY MAKI AND FOSTER (1957) ARE PROBABLY ALSO MEMBERS OF GENUS BUTYRIVIBRIO. GILL AND KING (1958) HAVE IDENTIFIED A CULTURE OF BUTYRIVIBRIO RECENTLY ISOLATED FROM THE INGESTA OF A BOVINE RUMEN.

ACCORDING TO THE ABOVE REPORTS, IT SEEMS THAT THE GENUS BUTYRIVIBRIO IS VERY COMMONLY DISTRIBUTED IN THE RUMEN AND IS ONE OF THE MOST NUMEROUS BACTERIA FROM CATTLE AND SHEEP FED A VARIETY OF RATIONS. HUNGATE (1950, 1952) HAS ISOLATED THEM FROM CATTLE IN WASHINGTON AND FROM CATTLE AND SHEEP IN NEW YORK. WILSON (1953) ISOLATED THEM FROM THE RUMEN OF SHEEP IN ENGLAND. BRYANT AND BUTKEY (1953B, C) ISOLATED THEM FROM CATTLE FED A WIDE VARIETY OF RATIONS INCLUDING ALFALFA HAY-GRAIN MIXTURE, ALFALFA HAY, GRAIN MIXTURE, WHEAT STRAW, ALFALFA SILAGE, FRESH ALFALFA AND BLUEGRASS PASTURE IN MARYLAND. THE SUB-GROUP B-1 OF MAKI AND FOSTER (1957) WAS OBTAINED IN CATTLE ON HIGH ROUGHAGE (ALFALFA HAY PLUS GRAIN) IN WISCONSIN. GILL AND KING (1958) FOUND IT IN CATTLE IN VIRGINIA. BUTYRIVIBRIO APPEARED IN LARGE NUMBERS AND WAS FREQUENTLY FOUND AS RATHER HIGH PERCENTAGE OF THE TOTAL ORGANISMS ISOLATED (BRYANT AND BURKEY, 1953B, C; MAKI AND FOSTER, 1957).

BRYANT AND SMALL (1956A, B) AND BRYANT (1956) WERE AMONG THE FIRST TO REPORT SYSTEMATIC CLASSIFICATION OF THE RUMEN BACTERIA. THEY USED FLAGELLATION, GRAM STAIN AND FERMENTATION PRODUCTS TO DIVIDE MOTILE CURVED RODS FOUND IN THE RUMEN INTO FOUR NEW GENERA INCLUDING BUTYRIVIBRIO. THE MORPHOLOGICAL CHARACTERISTICS OF THESE FOUR GENERA WERE RATHER SIMILAR. THERE WAS CONSIDERABLE VARIATION IN

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS BETWEEN STRAINS OF EACH GENUS, AND SOME OF THE STRAINS OF BUTYRIVIBRIO WERE VERY SIMILAR TO THE TYPE CULTURE OF OTHER GENERA. THE PRINCIPAL CHARACTERISTICS OF THE FOUR GENERA WHICH THEY DESCRIBED ARE AS FOLLOWS:

1. SUCCINIVIBRIO--ANAEROBIC, NONSPOREFORMING, GRAM NEGATIVE, CURVED RODS WITH MONOTRICHOUS POLAR FLAGELLATION. THIS GENUS FERMENTS GLUCOSE WITH THE PRODUCTION OF A LARGE AMOUNT OF SUCCINIC ACID AND LESSER AMOUNTS OF ACETIC, FORMIC AND LACTIC ACIDS.

2. LACHNOSPIRA--ANAEROBIC, NONSPOREFORMING, MONOTRICHOUS, WEAKLY GRAM POSITIVE, CURVED RODS THAT FERMENT GLUCOSE WITH THE PRODUCTION OF LARGE AMOUNTS OF ETHANOL LACTIC, FORMIC AND ACETIC ACIDS.

3. SELENOMONAS--ANAEROBIC, LARGE, CURVED, USUALLY CRESCENT-SHAPED RODS WITH TUFTS OF FLAGELLA OFTEN ATTACHED TO THE MIDDLE OF THE CONCAVE SIDE OF THE CELL. IT IS USUALLY GRAM NEGATIVE, BUT A FEW CELLS IN A SMEAR OFTEN APPEARED TO BE GRAM POSITIVE. ACIDS PRODUCED DURING GROWTH ON RUMEN FLUID GLUCOSE MEDIUM VARY. SOME STRAINS PRODUCED MAINLY LACTIC ACID AND SMALL AMOUNTS OF PROPIONIC AND ACETIC ACIDS. SOME STRAINS PRODUCED CHIEFLY PROPIONIC OR ACETIC ACIDS, WHILE ACIDS PRODUCED BY OTHER STRAINS LAY BETWEEN TWO EXTREMES. SMALL AMOUNTS OF BUTYRIC, FORMIC AND SUCCINIC ACIDS WERE DETECTABLE IN MOST CULTURES.

4. BUTYRIVIBRIO--ANAEROBIC, NONSPOREFORMING, MONO-TRICHOUS, GRAM NEGATIVE, CURVED RODS WHICH FERMENT GLUCOSE WITH THE PRODUCTION OF LARGE AMOUNTS OF BUTYRIC ACID. IT HAS BEEN NOTICED THAT UPON SUBCULTURE THE ORGANISM BECOMES LESS CURVED.

VARIOUS METHODS AND MEDIA HAVE BEEN USED FOR THE ISOLATION OF RUMEN BACTERIA, DEPENDING UPON THE INTERESTS OF THE INVESTIGATOR. AMONG THE MEDIA FOR THE ISOLATION OF RUMEN ORGANISMS, HUNGATE'S (1947) INORGANIC SALTS-SUGAR RUMEN FLUID AND THE ENRICHED ORGANIC MEDIUM OF HUHTANEN, ET AL. (1952) HAVE BEEN WIDELY USED. KING AND SMITH (1955) HAVE EVALUATED THESE TWO MEDIA AND CONCLUDED THAT THE INORGANIC SALTS, RUMEN FLUID, GLUCOSE, CELLULOSE MEDIUM WAS SUPERIOR TO THE HIGHLY ORGANIC MEDIUM FOR THE STUDY OF CELLULOLYTIC RUMEN BACTERIA. PROCEDURES SIMILAR TO THOSE OF HUNGATE WERE FOLLOWED BY SIJPESTEIEN (1951), BRYANT AND BURKEY (1953A) AND UNDERKOFER, ET AL. (1953). SINCE BRYANT AND HIS ASSOCIATES INTRODUCED THE FOUR NEW GENERA OF RUMEN BACTERIA MENTIONED ABOVE, THE PRESENT EXPERIMENTS CONCERNING BUTYRIVIBRIO WILL APPLY THEIR METHODS OF STUDY.

ACCORDING TO THE REPORTS OF BRYANT AND HIS GROUP, BUTYRIVIBRIO COULD EASILY BE ISOLATED AND DISTINGUISHED FROM THE OTHER ORGANISMS. THEY REPORTED THE ISOLATION OF 893 CULTURES FROM DIFFERENT RUMEN SAMPLES BY USING RUMEN FLUID, GLUCOSE, CELLOBIOSE, AND AGAR MEDIUM (RGCA) (BRYANT AND BURKEY, 1953A). THE MORPHOLOGICAL TYPES IN THE

BACTERIAL CULTURES INCLUDED VARIOUS COCCI, RODS, SPIRILLA AND SPIROCHETES. IN ANOTHER EXPERIMENT OF THE SAME REPORT, THEY POINTED OUT THAT IN A GLUCOSE OR CELLOBIOSE MEDIUM WITHOUT CARBONATE, ONLY THE GRAM NEGATIVE, MOTILE RODS (MR-GXCS AND MR-GXC) GAVE SIGNIFICANT AMOUNTS OF GROWTH. USING RGCA MEDIUM WITH A NITROGEN ATMOSPHERE AND WITHOUT SODIUM CARBONATE, IT SHOULD BE POSSIBLE TO ISOLATE GRAM NEGATIVE, MOTILE RODS, SINCE OTHER ORGANISMS WOULD BE INHIBITED. BY USE OF GRAM STAIN, LACHNOSPIRA ISOLATES (IF ANY) MAY BE DISCARDED SINCE LACHNOSPIRA GIVES A WEAKLY POSITIVE REACTION IN CONTRAST TO THE NEGATIVE REACTION OF BUTYRIVIBRIO. SUCCINIVIBRIO MAY BE DETECTED BY THE ABSENCE OF BUTYRIC ACID IN THE FERMENTATION PRODUCTS. SELENOMONAS CAN BE DISTINGUISHED FROM THE BUTYRIVIBRIO BY ITS TUFTED FLAGELLATION AS CONTRASTED WITH THE MONOTRICHOUS BUTYRIVIBRIO.

SINCE THE GENUS BUTYRIVIBRIO HAS ALWAYS BEEN FOUND TO BE AMONG THE MOST NUMEROUS BACTERIA AND BECAUSE OF THE WIDE VARIETY OF FEED CONSTITUENTS THAT IT ATTACKS, IT IS OF IMPORTANCE IN THE RUMEN. AS WE KNOW, A LARGE MICROBIAL POPULATION INCLUDING LARGE NUMBERS AND NUMEROUS KINDS OF BACTERIA IS FOUND IN THE RUMEN OF RUMINANTS. THE HOST SUPPLIES THE BACTERIAL FLORA WITH MATERIALS TO BE UTILIZED AND THE MICROBES IN TURN BREAK DOWN THESE SUBSTANCES TO SYNTHESIZE MANY NUTRITIOUS COMPOUNDS FOR THE HOST. NOTABLE AMONG FUNCTIONS OF THE BACTERIAL FLORA ARE THE DIGESTION OF

CELLULOSE, PRODUCTION OF FATTY ACIDS, AND THE SYNTHESIS OF PROTEINS AND VITAMINS. THESE FUNCTIONS WERE REVIEWED BY DOETSCH AND ROBINSON (1953). BRYANT AND SMALL (1956A) HAVE INDICATED THAT STRAINS OF BUTYRIVIBRIO DIGEST MANY OF THE MAJOR COMPONENTS OF RUMINANT RATIONS INCLUDING XYLAN, CELLULOSE, STARCH AND PROTEIN. THE CULTURE OF HUNGATE (1950) RAPIDLY UTILIZED A WIDE VARIETY OF CARBOHYDRATES INCLUDING HEMICELLULOSE, BUT ONLY SLIGHTLY DIGESTED CELLULOSE. THE ABILITY OF THE GENUS TO DIGEST THE CELLULOSE IS GRADUALLY LOST AFTER SUBCULTURE AS REPORTED BY HUNGATE (1950) AND BRYANT AND SMALL (1956A).

THE FERMENTATION PRODUCTS OF THE ORGANISM SEEM TO VARY BETWEEN STRAINS. HUNGATE'S (1950) LESS ACTIVELY CELLULOLYTIC ROD PRODUCED BUTYRIC, LACTIC AND FORMIC ACIDS WITH CARBON DIOXIDE, HYDROGEN AND TRACES OF ALCOHOL, AND UTILIZED SOME ACETIC AND PROPIONIC ACIDS. BRYANT AND SMALL (1956A) REPORTED THE FERMENTATION PRODUCTS OF TWO CULTURES. ONE CULTURE PRODUCED BUTYRIC, LACTIC, SUCCINIC, FORMIC, ACETIC AND PROPIONIC ACIDS WITH CARBON DIOXIDE, THE OTHER UTILIZED SOME ACETIC AND PRODUCED BUTYRIC, LACTIC AND FORMIC ACIDS, AND ETHANOL, HYDROGEN AND CARBON DIOXIDE. GILL AND KING (1958) HAVE STUDIED THE NUTRITIONAL CHARACTERISTICS OF A BUTYRIVIBRIO. ACCORDING TO THEIR OBSERVATION, THE CHARACTERISTIC FERMENTATION OF THEIR STRAIN OF THE ORGANISM WAS THE PRODUCTION OF A LARGE AMOUNT OF BUTYRIC ACID AND SOME

LACTIC ACID OR SUCCINIC ACID, WITH PRODUCTION OF LITTLE OR NO PROPIONIC ACID. THEY ALSO FOUND THAT WHEN RUMEN FLUID WAS OMITTED FROM THE FERMENTATION MEDIUM, THE PRODUCTION OF BUTYRIC ACID DECREASED MARKEDLY. IN ADDITION, THE UPTAKE OR PRODUCTION OF ACETIC ACID DEPENDED UPON THE MEDIUM USED. IT IS OBVIOUS THAT EVEN IN THE SAME STRAIN, FERMENTATION PRODUCTS MAY VARY WITH VARIATIONS IN THE MEDIUM. THIS IS THE POINT THAT LEADS TO THE STUDY OF THE FERMENTATION CHARACTERISTICS OF THIS GENUS IN THE PRESENT EXPERIMENT. BESIDES THE FATTY ACIDS, HUHTANEN AND GALL (1953) REPORTED THAT THE RO-H TYPE ORGANISMS SYNTHESIZED FOLIC ACID AND RIBOFLAVIN, AND RO-H₂ ALSO SYNTHESIZED BIOTIN.

SO FAR, WE STILL DO NOT KNOW THE EXACT MECHANISM USED BY RUMINANTS TO UTILIZE THE FATTY ACIDS THAT THE RUMEN BACTERIA PRODUCE. THE MANY STUDIES OF THE RUMEN PHYSIOLOGISTS INDICATE THE IMPORTANCE OF THESE ACIDS IN THE RUMEN. IT HAS BEEN KNOWN FOR SOME TIME THAT THE PRINCIPAL FATTY ACIDS DURING MICROBIAL FERMENTATION IN THE RUMEN ARE ACETIC, PROPIONIC AND BUTYRIC ACIDS. DIFFERENT ORDERS OF ABSORPTION RATES OF THESE THREE FATTY ACIDS FROM THE RUMEN WERE REPORTED BY MASSON AND PHILLIPSON (1951); PFANDER AND PHILLIPSON (1953) AND JOHNSON (1951). THE METABOLISM OF SHORT CHAIN FATTY ACIDS IN THE SHEEP WAS REPORTED BY PENNINGTON (1952). HE FOUND THAT BUTYRIC ACID WAS CONVERTED TO KETONE BODIES, MAINLY ACETOACETIC ACID. ACETIC FORMED SOME KETONE BODIES

BUT NOT TO THE SAME EXTENT AS DID BUTYRIC. IN ANOTHER PAPER, PENNINGTON AND SUTHERLAND (1956) FOUND THAT LACTATE WAS METABOLIZED WITH THE FORMATION OF KETONE BODIES, AND GLUCOSE LOWERED ENDOGENOUS KETONE BODY FORMATION. CLARK AND MALAN (1956), DOSING THE RUMEN OF SHEEP WITH ACETATE, PROPIONATE AND BUTYRATE, FOUND THE ACETATE CAUSED A SLIGHT AND DELAYED RISE IN KETONE BODIES WITHOUT AFFECTING THE BLOOD SUGAR, PROPIONATE PRODUCED A SHARP RISE IN BLOOD SUGAR, AND BUTYRATE PRODUCED A SHARP RISE IN BLOOD KETONES, MAINLY BETA-HYDROOXYBUTYRIC ACID, TOGETHER WITH A FALL IN BLOOD SUGAR. IN ADDITION, ACETOACETIC ACID INJECTED INTRAVENOUSLY WAS PARTIALLY CONVERTED TO BETA-HYDROOXYBUTYRIC ACID. GRAY, ET AL. (1952), WORKING WITH CARBOXYL-LABELLED ACETIC ACID, FOUND THAT RADIO-ACETIC ACID GAVE RISE, WHEN INCUBATED IN VITRO WITH RUMEN CONTENTS, TO RADIO-BUTYRIC, RADIO-VALETIC AND RADIO-PROPIONIC ACID. RADIO-PROPIONIC PRODUCED RADIO-VALERIC AND A TRACE OF RADIO-ACETIC BUT NO RADIO-BUTYRIC. THE RESULT SUGGESTED A SYNTHESIS OF THE HIGHER ACIDS BY CONDENSATION OF THE LOWER ONES WITH 2 C COMPOUNDS IN EQUILIBRIUM WITH ACETIC ACID. EMERY, ET AL. (1956) REPORTED THAT THE COW OBTAINED 3 TO 13 PER CENT OF ITS ENERGY FROM SHORT CHAIN ACIDS. KLEIBER, ET AL. (1952, 1954) REPORTED THAT BUTYRATE AND ACETATE WERE PRECURSORS OF MILK CONSTITUENTS IN THE INTACT DAIRY COW. HIGHER CHAIN FATTY ACIDS, SUCH AS N-VALERIC, ISOVALERIC, ETC., WERE

REPORTED BY BENTLEY, ET AL. (1954) TO STIMULATE CELLULOSE DIGESTION AND THE CONVERSION OF UREA NITROGEN INTO PROTEIN BY RUMEN BACTERIA. THAT THESE ACIDS WERE REQUIRED BY A RUMEN CELLULOLYTIC BACTERIUM, BACTEROIDES SUCCINOGENES, FOR GROWTH WAS INDICATED BY BRYANT AND DOETSCH (1955).

INTRODUCTION

HUNGATE (1950), HUHTANEN AND GALL (1952), WILSON (1953) AND MAKI AND FOSTER (1957) HAVE REPORTED ANAEROBIC, GRAM NEGATIVE, BUTYRIC ACID-PRODUCING CURVED RODS ISOLATED FROM THE RUMEN OF CATTLE AND SHEEP. BRYANT AND SMALL (1956A) WERE THE FIRST TO ESTABLISH A NEW GENUS, BUTYRIVIBRIO, FOR THOSE ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, CURVED RODS PRODUCING A LARGE AMOUNT OF BUTYRIC AND SOME LACTIC, FORMIC, ACETIC AND SUCCINIC ACIDS. GILL AND KING (1958) HAVE DESCRIBED THE NUTRITIONAL CHARACTERISTICS OF A BUTYRIVIBRIO. SINCE THE GENUS IS ABLE TO DIGEST A WIDE VARIETY OF FEED CONSTITUENTS WHICH MAY BE PRESENT DURING THE RUMEN FERMEN-TATION, AND THE FATTY ACIDS PRODUCED BY THIS AND OTHER RUMEN BACTERIA ARE ABSORBED BY RUMINANTS TO BE USED AS AN ENERGY SOURCE (EMERY, ET AL., 1956), AS PRECURSORS OF MILK CONSTITUENTS (KLEIBER, ET AL., 1952, 1954) AND OF KETONE BODIES (PENNINGTON, 1952, AND PENNINGTON AND SUTHER-LAND, 1956), THE FERMENTATION CHARACTERISTICS OF THIS GENUS ARE WORTHY OF SPECIAL ATTENTION.

MATERIALS AND METHODS

THE ANAEROBIC TECHNIQUE USED TO CULTURE THE RUMEN BACTERIA WAS THAT OF HUNGATE (1950). METHODS AND MEDIA FOR CULTURE ISOLATION AND STUDY OF PHYSIOLOGICAL CHARACTERISTICS WERE MAINLY THOSE OF BRYANT AND BURKEY (1953A), BRYANT AND DOETSCH (1954) AND BRYANT AND SMALL (1956A) WITH MINOR VARIATIONS IN THE COMPOSITION OF THE MEDIA AND THEIR PREPARATION.

CULTURE ISOLATION MEDIUM. CULTURES WERE ISOLATED FROM RUMEN INGESTA ON BRYANT AND BURKEY'S (1953A) ISOLATION MEDIUM WHICH WAS MODIFIED TO CONTAIN 15 ML. EACH OF THEIR MINERAL SOLUTIONS No. 1 AND No. 2, 0.4 GM. OF GLUCOSE, 0.1 ML. OF A 0.1 PER CENT SOLUTION OF RESAZURIN AND 40 ML. OF RUMEN FLUID (THE COMPOSITION OF BRYANT AND BURKEY'S MINERAL SOLUTIONS IS SHOWN IN APPENDIX 2). THE PH WAS ADJUSTED TO 6.9 AND THE TOTAL VOLUME WAS BROUGHT UP TO 97.75 ML. WITH WATER. EIGHT AND EIGHT-TENTHS ML. OF THE MEDIUM WAS DISPENSED TO TUBES CONTAINING 0.18 GM. AGAR. TWO TENTHS OF A ML. OF FRESHLY PREPARED CYSTEINE HYDROCHLORIDE SOLUTION CONTAINING 0.1 GM. OF CYSTEINE HYDROCHLORIDE IN 4.5 ML. OF WATER WAS THEN ADDED TO EACH TUBE AND THE TUBES WERE FLUSHED WITH NITROGEN. THE TUBES WERE THEN CLOSED WITH RUBBER STOPPERS WHICH WERE WIRED IN PLACE FOR STERILIZATION AT 121 C FOR 20 MIN. ALL TEST MEDIA USED IN THIS STUDY CONTAINED CYSTEINE HYDROCHLORIDE AND WERE PREPARED BY THE SAME METHOD AS FOR THE ISOLATION MEDIUM.

THE RUMEN FLUID USED IN THESE MEDIA WAS PREPARED BY THE METHOD OF GILL AND KING (1958), BUT THE FLASKS WERE FLUSHED WITH CARBON DIOXIDE BEFORE STERILIZATION AND STORAGE IN THE REFRIGERATOR.

SAMPLE DILUTION. SAMPLES OF RUMEN FLUID WERE DILUTED IN THE ANAEROBIC DILUTION MEDIUM OF BRYANT AND BURKEY (1953A). HOWEVER, SODIUM CARBONATE WAS OMITTED AND SODIUM HYDROXIDE WAS USED TO ADJUST THE PH. NITROGEN WAS BUBBLED THROUGH THE SOLUTION TO REPLACE ANY OXYGEN PRESENT.

SAMPLE TREATMENT, DILUTION SERIES, AND ROLL TUBE PREPARATION WERE ACCORDING TO THE METHODS DESCRIBED BY BRYANT AND BURKEY (1953A) EXCEPT THAT NITROGEN WAS USED INSTEAD OF CARBON DIOXIDE TO MAINTAIN ANAEROBIC CONDITIONS. THE TUBES WERE INCUBATED AT 39 C FOR 3 DAYS.

ISOLATION OF BACTERIA. ALL COLONIES FROM CULTURES INOCULATED WITH 1×10^{-8} DILUTION OF RUMEN SAMPLES WERE ISOLATED. PURE CULTURES WERE OBTAINED BY TWICE SERIALLY DILUTING THE WELL ISOLATED COLONIES IN TUBES OF THE ISOLATION MEDIUM AND PREPARING ROLL TUBE CULTURES. MACROSCOPIC AND MICROSCOPIC OBSERVATION OF SEVERAL COLONIES IN EACH TUBE VERIFIED THE PRESENCE OF SINGLE MORPHOTYPES. ISOLATED CULTURES WERE MAINTAINED ON THE RUMEN FLUID, GLUCOSE, CELLOBIOSE, AGAR (RGCA) MEDIUM OF BRYANT AND BURKEY (1953A) WITHOUT MODIFICATION.

CULTURAL TESTS. MEDIA AND METHODS FOR STUDIES OF ANAEROBIOSIS, MORPHOLOGY, MOTILITY, MINIMUM PH, TEMPERATURE,

VOGES-PROSKAUER TEST, HYDROGEN SULFIDE PRODUCTION, CARBOHYDRATE UTILIZATION, STARCH HYDROLYSIS, CELLULOSE DIGESTION, NITRATE REDUCTION, INDOL PRODUCTION, GELATIN LIQUEFACTION AND CASEIN DIGESTION WERE THOSE OF BRYANT AND DOETSCH (1954) AND BRYANT AND SMALL (1956A). UNLESS OTHERWISE STATED, THE TEMPERATURE, THE INOCULUM, THE GAS AND THE TIME OF INCUBATION WERE THE SAME AS REPORTED BY BRYANT AND HIS ASSOCIATES (1954, 1956A).

RUMEN FLUID REPLACEMENT. ATTEMPTS WERE MADE TO USE YEAST EXTRACT AND TRYPTICASE IN PLACE OF RUMEN FLUID IN A MODIFIED MEDIUM OF BRYANT AND SMALL (1956A). THE COMPOSITION OF THIS MEDIUM IS SHOWN IN APPENDIX 2. THE PH OF THIS MEDIUM AFTER STERILIZATION WAS 6.9.

MORPHOLOGY AND FERMENTATION CHARACTERISTICS. FLAGELLA STAINS WERE CARRIED OUT BY ADDING ONE-HALF ML. OF DISTILLED WATER TO 18 HR. SLANTS OF THE CULTURE GROWN ON RGCA MEDIUM (BRYANT AND SMALL, 1956A). THE RESULTING CELL SUSPENSIONS WERE WASHED TWICE WITH 1 ML. ALIQUOTS OF DISTILLED WATER BY CENTRIFUGATION AND EACH SUSPENSION VOLUME WAS MADE UP TO 1 ML. TUBES WERE CENTRIFUGED EACH TIME AT A 1680 X G FOR 10 MIN. SUSPENSIONS WERE STAINED BY LEIFSON'S METHOD FOR FLAGELLA STAINS.

CULTURE MORPHOLOGY AND FERMENTATION CHARACTERISTICS WERE DETERMINED IN THE RUMEN FLUID GLUCOSE MEDIUM OF BRYANT AND SMALL (1956A) CONTAINING 20 PER CENT RUMEN FLUID, MINERALS, 0.05 PER CENT CYSTEINE HYDROCHLORIDE, 0.4 PER CENT GLUCOSE

AND 0.2 PER CENT SODIUM BICARBONATE. THE MEDIUM WAS MODIFIED TO CONTAIN 0.0001 PER CENT RESAZURIN AND WAS ADJUSTED TO PH 6.9 BEFORE STERILIZATION UNDER A NITROGEN ATMOSPHERE. AFTER STERILIZATION, THE PH OF THE MEDIUM WAS 7.0. FOR THE STUDY OF MORPHOLOGICAL CHARACTERISTICS OF THE CULTURE, THIS MEDIUM WAS USED WITH A CARBON DIOXIDE ATMOSPHERE. THE MEDIUM WAS FLUSHED WITH NITROGEN TO STUDY THE FERMENTATION PRODUCTS OF THE CULTURES.

EFFECT OF AGE OF CULTURE. TO TEST THE EFFECT OF CULTURE AGE UPON THE CONCENTRATION OF FERMENTATION PRODUCTS, 180 ML. VOLUMES OF THE LATTER MEDIUM ABOVE WERE INOCULATED WITH 1 ML. OF 24 HR. CULTURE AND INCUBATED AT 39 C FOR 14 DAYS. SAMPLES OF THE CULTURES WERE TAKEN AT 24 HR. INTERVALS FOR ANALYSIS OF FATTY ACIDS. THE PH OF THIS MEDIUM AFTER STERILIZATION WAS 6.45. (THE RUMEN FLUID USED IN THIS EXPERIMENT WAS OBTAINED FROM ANOTHER STEER FED THE SAME RATION.)

EFFECT OF PH AND PRODUCT CONCENTRATION. FOR PH STUDIES THE RUMEN FLUID GLUCOSE MEDIUM CONTAINED 0.03 M K_2HPO_4 AND REQUIRED AMOUNTS OF CONCENTRATED H_3PO_4 . BEFORE STERILIZATION, THE BUFFERED PH VALUES FOR THESE MEDIA WERE 7.0, 6.5, 6.0 AND 5.5. AFTER STERILIZATION, THE PH VALUES WERE 7.07, 6.59, 6.10 AND 5.25 RESPECTIVELY.

ONE AND ELEVEN-HUNDREDTHS MILLIEQUIVALENTS OF ACETIC, BUTYRIC, PROPIONIC AND LACTIC ACIDS WERE ADDED PER 100 ML. OF MEDIUM IN EXPERIMENTS TO DETERMINE THE EFFECT OF PRODUCT

CONCENTRATION UPON FERMENTATION ACTIVITY. THE ACIDS WERE ADDED INDIVIDUALLY OR MIXED TO THE BUFFERED RUMEN FLUID MEDIUM. ALL THE MEDIA WERE ADJUSTED TO 6.5 BEFORE STERILIZATION. THE PH OF THESE MEDIA AFTER STERILIZATION WAS SHOWN IN TABLES 8, 9, 10 AND 11.

FERMENTATION IN RUMEN FLUID. A RUMEN FLUID MEDIUM WAS PREPARED BY CENTRIFUGATION OF FRESH RUMEN FLUID AT 20,000 X G FOR 30 MIN. ONE-HALF OF ONE PER CENT OF CASEIN HYDROLYZATE, 1.5 PER CENT OF GLUCOSE, 0.05 PER CENT OF CYSTEINE HYDROCHLORIDE AND 0.0001 PER CENT OF RESAZURIN WERE ADDED TO THE RUMEN FLUID SUPERNATANT. THE PH OF THIS MEDIUM WAS ADJUSTED TO 6.9 BEFORE STERILIZATION. NITROGEN OR CARBON DIOXIDE WAS USED TO FLUSH THE MEDIUM.

UNLESS OTHERWISE STATED, ALL FERMENTATION TESTS WERE INOCULATED WITH ONE DROP OF A 24 HR. CULTURE GROWN IN RUMEN FLUID GLUCOSE MEDIUM AND INCUBATED AT 37 C FOR 14 DAYS UNDER A NITROGEN ATMOSPHERE.

FOR IDENTIFICATION OF BUTYRIVIBRIO CULTURES, FATTY ACIDS WERE DETERMINED BY THE METHOD OF LANGSTON (1955). FOR OTHER FERMENTATION EXPERIMENTS, THEY WERE DETERMINED BY THE METHOD OF SMITH, ET AL. (1956).

RESULTS

DUPLICATE SAMPLES OF RUMEN INGESTA WERE TAKEN FROM THE TOP AND BOTTOM OF THE RUMEN OF A FISTULATED STEER FED A WINTER RATION OF POOR QUALITY ALFALFA HAY AND 4 LBS. OF GRAIN CONCENTRATE PER DAY. FOURTY-NINE ISOLATED COLONIES WERE PICKED FROM THE 1×10^{-8} DILUTION OF 8 ROLL TUBES. TEN OF THE ISOLATED COLONIES WERE ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, CURVED RODS, WHICH PRODUCED BUTYRIC, FORMIC, LACTIC, PROPIONIC AND SUCCINIC ACIDS. STRAINS DESIGNATED A AND C WERE OBTAINED FROM 2 TOP SAMPLES, AND D FROM 1 BOTTOM SAMPLE. STRAIN G WAS ISOLATED AND REPORTED BY GILL AND KING (1958). THERE WAS CONSIDERABLE VARIATION IN THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS BETWEEN THE 11 ISOLATES STUDIED. THE MORPHOLOGICAL CHARACTERISTICS OF THE ISOLATES ARE GIVEN IN TABLES 1 AND 2.

THE ORGANISMS GREW WELL IN RUMEN FLUID GLUCOSE MEDIUM WITH A NITROGEN ATMOSPHERE IN PLACE OF CARBON DIOXIDE AND WITH BICARBONATE OMITTED. SOME CULTURAL CHARACTERISTICS OF THE CULTURES ARE SHOWN IN TABLE 3. NO VISIBLE GROWTH OR PH REDUCTION WAS FOUND IN ANY CULTURE AT 50 C OR 22 C. THEY ALL GREW WELL AT 30 C AND 37 C. GROWTH WAS ALSO OBTAINED AT 45 C. NONE OF THE CULTURES PRODUCED ACETYL METHYL CARBINOL OR INDOL OR LIQUEFIED GELATIN. THE RESULTS OF H_2S PRODUCTION, CASEIN DIGESTION AND NITRATE REDUCTION VARIED BETWEEN STRAINS AS SHOWN IN TABLE 3. ALL OF THE

CULTURES PRODUCED ACID FROM XYLOSE, GLUCOSE, FRUCTOSE, SUCROSE, LACTOSE, CELLOBIOSE, RAFFINOSE AND SALICIN, BUT NONE OF THEM FERMENTED SORBITOL AND MANNITOL. ONLY 6 STRAINS OF THE 11 HYDROLYZED STARCH. NO VISIBLE LOSS OF THE CELLULOSE WAS FOUND FROM TUBES OF RUMEN FLUID CELLULOSE BROTH AFTER ONE WEEK OF INCUBATION.

THE ORGANISMS ALSO GREW WELL WHEN RUMEN FLUID WAS REPLACED BY YEAST EXTRACT AND TRYPTICASE IN THE GLUCOSE MEDIUM. THE FERMENTATION PRODUCTS OF THESE CULTURES ARE GIVEN IN TABLE 4.

CONCENTRATIONS OF FATTY ACIDS PRODUCED BY THE 11 STRAINS IN RUMEN FLUID GLUCOSE MEDIUM WERE DETERMINED BY TWO METHODS (LANGSTON, 1955 AND SMITH, ET. AL., 1956). RESULTS ARE SHOWN IN TABLES 5 AND 6.

TABLE 7 SHOWS THE FATTY ACID PRODUCTION AT DIFFERENT PH VALUES IN BUFFERED RUMEN FLUID GLUCOSE MEDIUM. ONLY STRAIN A4 GREW AT PH 5.25.

EFFECTS OF ADDITION OF ACIDS TO THE BUFFERED MEDIUM ARE RECORDED IN TABLES 8, 9, 10 AND 11. THE ADDED FATTY ACID HAS ALREADY BEEN SUBTRACTED FROM THE RESULTS IN THESE TABLES. THE UNDERLINED FIGURES INDICATE THE FATTY ACID WHICH WAS ADDED IN EACH TREATMENT.

STRAINS A4 AND G HAVE BEEN USED TO TEST THE EFFECT OF AGE OF CULTURE UPON FERMENTATION PRODUCTS. THE RESULTS OF THE ANALYSES OF FATTY ACIDS PRODUCED AT 24-HOUR INTERVALS BY THESE TWO CULTURES ARE GIVEN IN TABLES 12 AND 13.

STRAINS A4, C7, C15 AND G WERE CULTURED IN THE 98 PER
CENT RUMEN FLUID MEDIUM. THE RESULTS OF FATTY ACID ANALYSIS
ARE GIVEN IN TABLE 14 AND 15.

TABLE I. MORPHOLOGICAL AND COLONIAL CHARACTERISTICS OF THE 11 STRAINS OF BUTYRIVIBRIO GROWN IN RGCA SLANTS

STRAIN	SHAPE	CELLS (18 HR) OCCURRENCE	SIZE (μ)	FLAGELLUM ^M		COLONIES (72 HR.)	
				WAVE LENGTH (μ)	AMPLITUDE (μ)	APPEARANCE	DIAMETER (MM.)
A4	C.R.	SINGLE AND SHORT CHAINS	0.3-0.4 x 1.4-2.7	2.11	0.38	TRANSLUCENT LIGHT TAN	1.0
C5	S.C.R.	"	0.2-0.4 x 1.4-2.4	2.41	0.53	"	0.5
C7	V.S.C.R.	"	"	1.98	0.33	"	IRREG.
C8	"	"	0.3-0.5 x 2.0-2.7	2.44	0.41	"	0.5-1.0
C9	"	"	0.3-0.4 x 1.4-3.4	2.38	0.40	"	IRREG.
C13	"	"	0.2-0.3 x 1.7-2.4	1.98	0.36	"	1.0-2.0
C15	"	LONG CHAINS	0.6-0.7 x 2.7-5.4	1.98	0.33	LIGHT TAN	1.0-2.0
D3	"	SINGLE, SHORT & LONG CHAINS	0.6-0.7 x 2.4-4.0	2.64	0.61	FILAMENTOUS TAN	1.0
D5	"	SHORT AND LONG CHAINS	0.3-0.4 x 2.0-2.7	1.98	0.33	"	1.0-1.5
D11	"	LONG CHAINS	0.3-0.5 x 5.7-6.7	1.65	0.26	"	2.0-2.5
G	S.C.R.	SINGLE AND SHORT CHAINS	0.3-0.4 x 2.4-3.0	1.91	0.33	TRANSLUCENT LIGHT TAN	IRREG.

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C = CURVED, R = RODS, S = SLIGHT, V = VERY

^MGLUCOSE CONCENTRATION OF THE MEDIUM REDUCED TO 0.1 PER CENT AND THE RESULTS WERE OBTAINED FROM 18 HR SLANTS OF RGCA.

TABLE 2. MORPHOLOGICAL CHARACTERISTICS OF THE 11 STRAINS OF
BUTYRIVIBRIO GROWN IN RUMEN FLUID GLUCOSE
 BROTH MEDIUM (24 HOUR CULTURES)

STRAIN	SHAPE	OCCURRENCE	SIZE (μ)	APPEARANCE
A4	S.C.R.	SINGLE AND SHORT CHAINS	0.3-0.4 x 2.0-2.7	TURBID
C5	V.S.C.R.	"	0.2-0.3 x 1.4-2.0	"
C7	"	"	0.2-0.4 x 1.4-2.0	"
C8	"	"	0.3-0.4 x 1.4-2.0	"
C9	"	"	0.3-0.4 x 1.2-1.7	"
C13	"	"	0.3-0.4 x 1.4-2.0	"
C15	"	SINGLE, SHORT & LONG CHAINS	0.4-0.5 x 2.7-3.4	FLOCCULENT SEDIMENT
D3	"	"	0.4-0.5 x 1.7-3.4	GRANULAR SEDIMENT ^x FILAMENTOUS MARGINS
D5	"	"	0.3-0.4 x 2.0-2.7	"
D11	"	"	0.3-0.5 x 1.4-3.4	"
G	S.C.R.	SINGLE AND SHORT CHAINS	0.3-0.4 x 1.7-2.7	TURBID

^xSOME OF WHICH ADHERED TO THE SIDES OF THE TUBE.

TABLE 3. SOME VARIABLE CHARACTERISTICS OF THE 11 STRAINS OF BUTYRIVIBRIO

	STRAIN											
	A4	C5	C7	C8	C9	C13	C15	D3	D5	D11	G	
H ₂ S PRODUCTION	SL.	-	+	-	-	-	-	-	-	-	-	-
CASEIN DIGESTION	-	-	-	-	-	-	-	-	-	-	-	+
NITRATE REDUCTION	+	-	-	-	-	-	-	-	-	-	-	-
HYDROLYSIS OF STARCH	+	-	-	-	-	-	+	+	+	+	+	+
FINAL PH OF MEDIUM	5.07	5.30	5.25	5.27	5.20	5.30	5.25	5.16	5.13	5.14	5.40	

SL. = SLIGHT

TABLE 4. FATTY ACIDS PRODUCED IN A YEAST EXTRACT AND TRYPTICASE MEDIUM BY THE STRAINS OF BUTYRIVIBRIO (14 DAY CULTURES)

STRAIN	ACID PRODUCED (MEQ/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	2.15	3.04	2.06	0.33
C5	1.62	0.06	0.02	1.33
C7	1.46	1.67	1.20	0.45
C8	1.72	0.17	0.36	2.73
C9	2.32	1.30	0.89	0.60
C13	1.84	0.06	0.22	2.28
C15	3.00	0.15	0.15	2.63
D3	2.80	0.00	0.77	1.95
D5	1.70	0.15	0.18	2.01
D11	2.42	0.05	0.79	2.93
G	1.00	0.00	0.37	3.88

TABLE 5. FATTY ACIDS PRODUCED IN A RUMEN FLUID GLUCOSE MEDIUM BY THE STRAINS OF BUTYRIVIBRIO DETERMINED BY THE METHOD OF LANGSTON (1955) (14 DAY CULTURES)

STRAIN	ACID PRODUCED (MEQ/100 ML)						
	BUTYRIC	PROPIONIC	ACETIC	FORMIC	SUCCINIC	LACTIC	TOTAL
A4	1.11	0.73	-0.84	0.64	0.05	0.05	1.74
C5	0.32	-0.09	-1.42	0.31	0.00	0.30	-0.58
C7	1.52	1.05	-0.82	0.85	0.15	-0.26	2.49
C8	0.32	-0.35	-1.43	0.71	0.00	0.10	-0.65
C9	1.21	-0.10	-1.50	0.10	0.02	0.89	0.62
C13	0.59	-0.25	-0.94	0.23	0.02	0.70	0.35
C15	0.74	0.04	-0.92	0.77	0.17	0.63	1.43
D3	0.59	-0.12	-0.46	0.61	0.00	0.28	0.90
D5	0.85	-0.25	-0.90	0.56	0.00	0.08	0.34
D11	1.44	-0.24	-1.05	0.78	0.04	0.01	0.98
G	0.39	0.12	-0.74	0.18	0.00	1.48	1.43
AVERAGE	0.83	0.05	-1.00	0.52	0.04	0.38	0.82
BLANK VALUES FOR MEDIUM	0.95	0.98	2.13	0.75	0.21	0.82	

TABLE 6. FATTY ACIDS PRODUCED IN A RUMEN FLUID GLUCOSE MEDIUM BY THE STRAINS OF BUTYRIVIBRIO ESTIMATED BY THE METHOD OF SMITH, ET. AL. (1956) (14 DAY CULTURES)

STRAIN	ACID PRODUCED (MEq/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	1.21	0.81	-0.15	0.40
C5	0.55	-0.10	-0.84	0.74
C7	1.59	1.13	0.00	-0.13
C8	0.48	-0.10	-0.50	0.41
C9	1.40	0.00	-1.24	1.00
C13	0.28	0.00	-0.41	1.10
C15	0.91	0.02	0.12	0.95
D3	0.48	-0.13	-0.05	0.52
D5	0.90	0.00	-0.15	0.57
D11	1.59	0.00	0.10	0.50
G	0.29	0.25	0.25	1.31
AVERAGE	0.88	0.17	-0.26	0.67

TABLE 7. FATTY ACIDS PRODUCED IN BUFFERED RUMEN FLUID
GLUCOSE MEDIA BY 11 STRAINS OF BUTYRIVIBRIO
(14 DAY CULTURES)

STRAIN	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	7.07	0.60	0.95	0.65	1.41
	6.59	0.95	0.98	0.57	0.55
	6.10	1.36	1.12	-0.37	0.18
	5.25	0.18	0.10	-0.36	0.10
C5	7.07	0.52	-0.02	0.17	2.72
	6.59	0.60	-0.01	0.08	1.83
	6.10	0.49	0.03	-0.19	1.38
C7	7.07	0.43	1.34	0.74	1.53
	6.59	1.13	0.87	0.01	0.53
	6.10	1.80	1.19	-0.54	0.13
C8	7.07	0.52	-0.03	0.09	2.52
	6.59	0.72	-0.02	-0.42	3.01
	6.10	0.38	0.10	-0.11	1.25
C9	7.07	0.60	-0.04	-0.22	1.98
	6.59	1.39	0.06	-0.82	2.98
	6.10	0.80	0.08	-0.73	1.59
C13	7.07	0.54	0.01	0.18	2.46
	6.59	0.78	0.02	-0.21	2.26
	6.10	0.58	-0.02	-0.24	1.78
C15	7.07	1.00	0.10	0.39	3.22
	6.59	1.69	0.12	0.05	1.80
	6.10	1.52	0.08	-0.44	1.08
D3	7.07	1.16	0.01	0.40	1.88
	6.59	1.70	0.07	0.15	1.65
	6.10	1.23	-0.02	-0.35	1.35
D5	7.07	1.42	-0.01	0.43	1.41
	6.59	1.52	0.13	-0.58	1.13
	6.10	1.41	-0.02	-0.22	1.04
D11	7.07	1.02	-0.04	0.37	1.92
	6.59	1.79	-0.07	0.36	1.51
	6.10	1.22	0.09	-0.40	1.15
G	7.07	0.39	0.43	0.07	2.16
	6.59	0.71	0.13	-0.15	2.36
	6.10	0.70	0.14	-0.50	1.28

TABLE 8. FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS IN A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN A4^x (14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>1.20</u>	0.85	0.33	0.78
2	6.56	1.76	<u>0.25</u>	0.57	0.68
3	6.55	1.29	0.81	<u>0.19</u>	0.97
4	6.55	1.33	1.40	0.63	<u>-0.03</u>
5	6.35	<u>1.37</u>	<u>1.14</u>	<u>0.46</u>	<u>0.04</u>
CONTROL	6.35	1.23	0.90	0.21	0.75

^xTHE UNDERLINED VALUE IN EACH TREATMENT INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEQ/100 ML.

TABLE 9. FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS IN A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN C7* (14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>1.29</u>	0.79	0.00	1.13
2	6.56	1.74	<u>1.00</u>	-0.39	0.38
3	6.55	1.52	1.18	<u>-0.45</u>	0.23
4	6.55	1.18	1.56	0.03	<u>-0.39</u>
5	6.35	<u>1.52</u>	<u>1.21</u>	<u>0.22</u>	<u>-0.33</u>
CONTROL	6.35	1.34	0.96	-0.12	0.67

*THE UNDERLINED VALUE IN EACH TREATMENT INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEQ/100 ML.

TABLE 10. FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS IN A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN G^x (14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>0.79</u>	0.02	-0.52	2.99
2	6.56	1.47	<u>0.12</u>	-0.44	1.75
3	6.55	1.21	0.02	<u>-0.65</u>	1.79
4	6.55	1.17	0.01	-0.62	<u>2.55</u>
5	6.35	<u>0.40</u>	<u>0.06</u>	<u>-0.45</u>	<u>2.76</u>
CONTROL	6.35	0.76	0.08	-0.93	1.70

^xTHE UNDERLINED VALUE IN EACH TREATMENT INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEQ/100 ML.

TABLE II. FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS IN A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN C15^x (14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>1.46</u>	0.07	0.09	2.19
2	6.56	1.78	<u>-0.15</u>	0.06	1.41
3	6.55	1.91	0.02	<u>-0.77</u>	1.61
4	6.55	1.71	-0.04	-0.14	<u>2.80</u>
5	6.35	<u>1.96</u>	<u>0.06</u>	<u>-0.62</u>	<u>2.24</u>
CONTROL	6.35	1.55	0.02	-0.34	1.44

^xTHE UNDERLINED VALUE IN EACH TREATMENT INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEQ/100 ML.

TABLE 12. THE EFFECT OF TIME OF INCUBATION ON THE PRODUCTION OF FATTY ACID BY STRAIN A4

DAY	ACID PRODUCED (MEQ/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
1	1.58	0.03	-0.71	1.22
2	1.63	0.24	-0.75	1.10
3	1.70	0.30	-0.69	0.95
4	1.83	0.47	-0.63	0.75
5	1.45	0.51	-0.73	0.75
6	1.72	0.46	-0.68	0.66
7	1.55	0.60	-0.52	1.02
8	1.48	0.67	-0.27	1.38
9	1.40	0.63	-0.31	0.99
10	1.45	0.57	-0.17	1.69
11	1.59	0.63	-0.15	1.31
12	1.84	0.75	-0.20	0.80
13	1.83	0.61	-0.33	0.62
14	1.93	0.60	-0.10	0.72

TABLE 13. THE EFFECT OF TIME OF INCUBATION ON THE PRODUCTION OF FATTY ACID BY STRAIN G

DAY	ACID PRODUCED (MEq/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
1	-0.02	0.15	-0.30	0.82
2	0.08	0.12	-0.41	1.22
3	0.10	0.16	-0.29	1.32
4	0.20	0.15	-0.44	1.55
5	0.17	0.16	-0.55	1.15
6	0.43	0.39	-0.34	0.98
7	0.33	0.66	-0.31	0.76
8	0.40	0.75	0.33	1.55
9	0.44	0.96	0.30	1.04
10	0.46	1.06	0.32	1.75
11	0.41	1.23	0.32	1.15
12	0.40	1.21	0.36	0.92
13	0.45	1.22	0.56	0.41
14	0.36	1.22	0.57	0.66

TABLE 14. FATTY ACIDS PRODUCED IN A 98 PER CENT RUMEN FLUID MEDIUM BY STRAINS OF BUTYRIVIBRIO UNDER NITROGEN ATMOSPHERE (14 DAY CULTURES)

STRAIN	ACID PRODUCED (MEq/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	1.77	0.39	-1.05	0.77
C7	4.65	1.22	-4.15	-1.08
C15	3.47	0.57	-2.60	0.73
G	2.19	0.00	-1.12	1.46

TABLE 15. FATTY ACIDS PRODUCED IN A 98 PER CENT RUMEN FLUID MEDIUM BY STRAINS OF BUTYRIVIBRIO UNDER CARBON DIOXIDE ATMOSPHERE (14 DAY CULTURES)

STRAIN	ACID PRODUCED (MEq/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	3.38	0.15	-2.15	1.26
C7	5.40	1.38	-4.06	-0.88
C15	3.51	0.56	-1.11	2.41
G	3.32	0.32	-2.51	0.89

TABLE 16. PERCENTAGE OF THE FATTY ACIDS PRODUCED IN BUFFERED RUMEN FLUID GLUCOSE MEDIA BY 11 STRAINS OF BUTYRIVIBRIO

STRAIN	BUFFERED PH	TOTAL ACIDS PRODUCED MEQ/100 ML.	BUTYRIC %	PROPIONIC %	ACETIC PLUS FORMIC %	SUCCINIC PLUS LACTIC %
A4	7.07	3.61	16.62	26.31	18.00	39.05
	6.59	3.05	31.15	32.13	18.69	18.03
	6.10	2.29	51.13	42.10	(-)	6.76
	5.25	0.02	47.37	26.31	(-)	26.31
C5	7.07	3.39	15.25	(-)	4.98	79.76
	6.59	2.50	23.90	(-)	3.19	72.91
	6.10	1.71	25.79	1.58	(-)	72.63
C7	7.07	4.04	10.64	33.17	18.32	37.87
	6.59	2.54	44.49	34.25	0.39	20.86
	6.10	2.58	57.69	38.14	(-)	4.17
C8	7.07	3.10	16.61	(-)	2.87	80.51
	6.59	3.29	19.30	(-)	(-)	80.69
	6.10	1.62	21.96	5.78	(-)	72.25
C9	7.07	2.32	23.25	(-)	(-)	76.74
	6.59	3.61	31.38	1.35	(-)	67.27
	6.10	1.74	32.39	3.24	(-)	64.37
C13	7.07	3.19	16.93	0.31	5.64	77.11
	6.59	2.85	25.49	0.65	(-)	73.85
	6.10	2.10	24.57	(-)	(-)	75.42
C15	7.07	4.71	21.23	2.12	8.28	68.36
	6.59	3.66	46.17	3.27	1.37	49.18
	6.10	2.24	56.71	2.98	(-)	40.30
D3	7.07	3.45	33.62	0.29	11.59	54.49
	6.59	3.57	47.61	1.96	4.20	46.23
	6.10	2.21	47.67	(-)	(-)	52.32
D5	7.07	3.25	43.56	(-)	13.19	43.25
	6.59	2.20	54.67	4.68	(-)	40.65
	6.10	2.21	57.55	(-)	(-)	42.45
D11	7.07	3.27	30.81	(-)	11.18	58.00
	6.59	3.59	48.90	(-)	9.84	41.26
	6.10	2.06	49.59	3.66	(-)	46.75
G	7.07	3.05	12.79	14.10	2.29	70.82
	6.59	3.05	22.19	4.06	(-)	73.75
	6.10	1.62	33.02	6.60	(-)	60.38

DISCUSSION AND CONCLUSION

TEN STRAINS OF ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, CURVED RODS HAVE BEEN OBTAINED FROM A TOTAL 49 RUMEN ISO-LATES. THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE STRAINS WERE STUDIED. MOST OF THE STRAINS PRODUCED A LARGE AMOUNT OF BUTYRIC AND SOME LACTIC, FORMIC AND PROPIONIC ACIDS WITH THE UTILIZATION OF ACETIC ACID IN A RUMEN FLUID GLUCOSE MEDIUM. A FEW OF THEM PRODUCED MORE LACTIC THAN BUTYRIC ACID. SINCE THE CLASSIFICATION OF THOSE ANAEROBIC, GRAM NEGATIVE, CURVED RODS FOUND IN THE RUMEN WAS MAINLY BASED ON GRAM REACTION, FLAGELLATION AND FERMENTATION PRODUCTS (BRYANT AND SMALL, 1956A), THE PRESENT STRAINS SHOULD BE THE MEMBERS OF BUTYRIVIBRIO. COMPARED TO THE TYPE CULTURE REPORTED BY BRYANT AND SMALL (1956A), THE PRESENT STRAINS HAVE THE IDENTICAL CHARACTERISTICS IN THAT THEY FERMENT XYLOSE, FRUCTOSE, GLUCOSE, CELLOBIOSE, SUCROSE, LACTOSE AND SALICIN AND DO NOT FERMENT MANNITOL, INOSITOL AND CELLULOSE. THERE WERE VARIATIONS OF THE OTHER CHARACTERISTICS SUCH AS CASEIN DIGESTION, HYDROGEN SULFIDE PRODUCTION AND NITRATE REDUCTION. SIMILAR VARIATION ALSO OCCURRED BETWEEN STRAINS DESCRIBED BY BRYANT AND SMALL (1956A).

THE RATIO OF FERMENTATION ACIDS WAS FOUND TO BE DEPENDENT UPON THE PH OF THE MEDIUM. GENERALLY SPEAKING, THERE IS A TENDENCY IN RELATIVELY HIGH PH MEDIA FOR CULTURES TO PRODUCE HIGHER AMOUNTS OF THE ACETIC (PLUS FORMIC) AND LACTIC (PLUS

SUCCINIC) ACIDS THAN AT A LOWER PH VALUE. ON THE OTHER HAND, THE BUTYRIC AND PROPIONIC ACIDS ARE FORMED IN HIGHER PERCENTAGES IN LOW PH MEDIA AND LOWER PERCENTAGES AT A HIGH PH.

AFTER THE SINGLE ADDITION OF ACETIC ACID OR PROPIONIC ACID TO THE MEDIUM, ALL FOUR TESTED STRAINS SHOWED AN INCREASE OF BUTYRIC ACID PRODUCTION COMPARED TO THE CONTROL. AFTER SINGLE ADDITION OF BUTYRIC ACID TO THE MEDIUM, AN INCREASE OF LACTIC AND A SLIGHT DECREASE OF BUTYRIC ACID WERE OBSERVED. AFTER SINGLE ADDITION OF LACTIC ACID, STRAINS A4 AND C7 PRODUCED A LARGE AMOUNT OF PROPIONIC ACID, BUT STRAINS G AND C15 PRODUCED A LARGE AMOUNT OF LACTIC ACID. WHEN ALL THE FOUR FATTY ACIDS WERE ADDED TO THE MEDIUM, STRAIN A4 AND C7 SHOWED AN INCREASE OF BUTYRIC, PROPIONIC AND ACETIC ACIDS, BUT A DECREASE OF LACTIC ACID. STRAIN G SHOWED A DECREASE OF BUTYRIC AND AN INCREASE OF LACTIC ACID. STRAIN C15 SHOWED AN INCREASE OF BUTYRIC AND LACTIC ACIDS. THE DATA INDICATE THAT THESE ORGANISMS MAY BE ACTIVE IN THE CONVERSION OF ACETATE AND POSSIBLY PROPIONATE TO BUTYRATE.

IN A 98 PER CENT RUMEN FLUID MEDIUM, ALL OF THE FOUR TESTED STRAINS SHOWED AN UPTAKE OF ACETIC (PLUS FORMIC) AND PRODUCED A LARGE AMOUNT OF BUTYRIC ACID. STRAIN C7 UTILIZED LACTIC (PLUS SUCCINIC) ACID AND PRODUCED A HIGH AMOUNT OF PROPIONIC ACID. ACCORDING TO THE RESULTS GIVEN

IN TABLES 14 AND 15, ALL THE STRAINS PRODUCED MORE FATTY ACIDS UNDER CARBON DIOXIDE ATMOSPHERE THAN UNDER NITROGEN IN THE SAME MEDIUM. THIS RESULT MAY SIMPLY REFLECT THE HIGH REQUIREMENT FOR CARBON DIOXIDE SUCH AS REPORTED FOR STRAIN G BY GILL AND KING (1958).

OTHER EVIDENCE WAS OBTAINED THAT THE HIGH AMOUNTS OF PROPIONIC ACID PRODUCED BY SOME STRAINS PROBABLY AROSE THROUGH UTILIZATION OF LACTIC ACID. STRAINS A4 AND C7 PRODUCED RATHER HIGH AMOUNTS OF PROPIONIC ACID, BUT THE LACTIC ACID WAS RATHER LOW. STRAINS G AND C9 PRODUCED LITTLE OR NO PROPIONIC BUT LARGE AMOUNTS OF LACTIC ACID. HOWEVER, IN SOME INSTANCES THEY BOTH PRODUCED RELATIVELY HIGH AMOUNTS OF PROPIONIC ACID AND RELATIVELY LOW AMOUNTS OF LACTIC ACID. THE EXACT MECHANISM BY WHICH THE STRAINS SHIFT THIS FERMENTATION REMAINS TO BE DETERMINED. THE FACT THAT THEY ARE SENSITIVE TO NEARLY ALL ENVIRONMENTAL CHANGES WILL COMPLICATE STUDIES OF THE MECHANISM BUT MAY IN PART EXPLAIN WHY FACTORS WHICH CONTROL RUMEN FERMENTATION IN VIVO HAVE BEEN SO ELUSIVE IN ANIMAL STUDIES. THE SATISFACTORY GROWTH ON A MEDIUM FROM WHICH RUMEN FLUID WAS OMITTED MIGHT INDICATE THAT BUTYRIVIBRIO ARE NOT AS FASTIDIOUS AS SEVERAL OTHER RUMEN ORGANISMS REPORTED TO DATE. IN THIS MEDIUM, BUTYRIC ACID WAS PRODUCED IN MUCH LARGER QUANTITIES AND THE ACETIC-FORMIC GROUP WAS INCREASED RATHER THAN DECREASED AS WITH THE OTHER MEDIA. THE AVERAGE RATIO

OF THE FATTY ACIDS PRODUCED BY ALL OF THE ISOLATES ON TRYPTICASE YEAST EXTRACT MEDIUM WAS APPROXIMATELY 4:1:1:4 FOR BUTYRIC, PROPIONIC, ACETIC AND FORMIC, AND LACTIC AND SUCCINIC RESPECTIVELY. ON RUMEN FLUID GLUCOSE MEDIUM AT PH 7.0 THE RATIO WAS 5:1:(-):1.5:4. IT SEEMS THAT THE HIGHER RATIO OF THE BUTYRIC ACID IN RUMEN FLUID GLUCOSE MEDIUM THAN IN THE YEAST EXTRACT AND TRYPTICASE MEDIUM MIGHT BE DUE IN PART TO BUTYRIC ACID PRODUCTION FROM CONDENSATION OF ACETIC ACID. THE RESULT MAY INDICATE THAT BUTYRIVIBRIO IS IMPORTANT IN THE PRODUCTION OF RADIO-BUTYRIC ACID DURING INCUBATION OF THE CARBOXYL-LABELLED ACETIC ACID IN VITRO WITH RUMEN CONTENTS REPORTED BY GRAY, ET AL. (1952).

SUMMARY

TEN STRAINS OF ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, BUTYRIC ACID-PRODUCING CURVED RODS HAVE BEEN ISOLATED FROM INGESTA OF THE BOVINE RUMEN AND IDENTIFIED AS MEMBERS OF BUTYRIVIBRIO. THESE TEN STRAINS OF BUTYRIVIBRIO REPRESENTED 1/5 OF ALL ISOLATES AT 1×10^{-8} DILUTIONS. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE TEN STRAINS AND STRAIN G, ISOLATED BY GILL AND KING (1958), HAVE ALSO BEEN STUDIED. MOST OF THE ORGANISMS PRODUCED A LARGE AMOUNT OF BUTYRIC AND SOME LACTIC, FORMIC AND PROPIONIC ACIDS WITH THE UTILIZATION OF ACETIC ACID IN A RUMEN FLUID GLUCOSE MEDIUM.

THE FERMENTATION CARRIED ON BY THESE ORGANISMS WAS SENSITIVE TO MOST TESTED ENVIRONMENTAL CHANGES. WHEN YEAST EXTRACT AND TRYPTICASE REPLACED RUMEN FLUID IN THE MEDIUM, THERE WAS A NET PRODUCTION OF THE ACETIC-FORMIC GROUP RATHER THAN A DECREASE. STUDIES WITH BUFFERED RUMEN FLUID GLUCOSE MEDIA HAVE SHOWN A SHIFT OF THE FERMENTATION PRODUCTS WITH PH. ADDITION OF FATTY ACIDS TO THIS MEDIUM, LEAD TO THE CONCLUSION THAT THESE ORGANISMS MAY BE ACTIVE IN THE CONVERSION OF ACETATE AND POSSIBLY PROPIONATE TO BUTYRATE. TWO STRAINS APPARENTLY HAVE THE ABILITY TO PRODUCE PROPIONATE AT THE EXPENSE OF LACTATE. THE RESULTS OF THE FERMENTATION TESTS OF 98 PER CENT RUMEN FLUID MEDIUM SHOWED THAT THE

TESTED STRAINS USED ACETIC (PLUS FORMIC) OR LACTIC (PLUS SUCCINIC) TO PRODUCE BUTYRIC OR PROPIONIC ACID AND PRODUCED HIGHER CONCENTRATIONS OF FATTY ACIDS UNDER A CARBON DIOXIDE ATMOSPHERE THAN UNDER NITROGEN.

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THE WRITER WISHES TO EXPRESS HIS DEEP APPRECIATION AND GREAT INDEBTEDNESS TO DR. W. E. C. MOORE FOR HIS KIND ASSISTANCE AND PATIENCE THROUGH ALL STAGES OF THE WORK LEADING TO THIS MANUSCRIPT, TO DRs. K. W. KING AND J. W. GILL FOR THEIR COURTESY TO SUPPLY THE STRAIN G OF BUTYRIVIBRIO AND VALUABLE SUGGESTIONS, AND TO DR. M. P. BRYANT FOR HIS KIND SUGGESTION WITH REFERENCE CONCERNING THE METHOD OF FLAGELLA STAIN.

APPENDIX I

THE FOLLOWING MANUSCRIPT DESCRIBING MAJOR PORTIONS OF THIS
THESIS WORK IS TO BE SUBMITTED TO THE EDITOR OF THE
JOURNAL OF BACTERIOLOGY.

ISOLATION AND FERMENTATION CHARACTERISTICS OF
BUTYRIVIBRIO SPECIES FROM RUMINAL INGESTA

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ANAEROBIC, GRAM NEGATIVE, BUTYRIC ACID-PRODUCING, CURVED RODS HAVE BEEN ISOLATED FROM THE RUMEN OF CATTLE AND SHEEP BY HUNGATE (1950), HUHTANEN AND GALL (1952), WILSON (1953), MAKI AND FOSTER (1957), AND GILL AND KING (1958). BRYANT AND SMALL (1956) ESTABLISHED A NEW GENUS FOR THESE ORGANISMS, BUTYRIVIBRIO. ORGANISMS OF THIS GENUS PROBABLY CONTRIBUTE SIGNIFICANTLY TO THE RUMEN FERMENTATION SINCE THEY HAVE BEEN ISOLATED BY SEVERAL INVESTIGATORS IN DIFFERENT AREAS OF THE COUNTRY, THEY OCCUR IN RELATIVELY HIGH NUMBERS IN RUMEN INGESTA, THEY ARE ABLE TO FERMENT A VARIETY OF SUBSTRATES NORMALLY PRESENT IN THE RUMEN, AND THEY PRODUCE CONSIDERABLE AMOUNTS OF FATTY ACIDS.

THE FERMENTATION CHARACTERISTICS OF THE TYPE CULTURE IN A DEFINED RUMEN FLUID-GLUCOSE MEDIUM WERE REPORTED BY BRYANT AND SMALL (1956). IN VIEW OF THE APPARENT IMPORTANCE OF THIS GENUS AN ATTEMPT WAS MADE, IN THE PRESENT INVESTIGATION, TO EXTEND THE OPERATIONS OF BRYANT ON THE INCIDENCE OF THE GENUS AND TO DETERMINE THE INFLUENCE OF ENVIRONMENTAL CHANGES WHICH ARE KNOWN TO TAKE PLACE IN THE RUMEN UPON THE FERMENTATION CHARACTERISTICS OF THIS GENUS.

MATERIALS AND METHODS

THE ANAEROBIC TECHNIQUE USED TO CULTURE THE RUMEN BACTERIA WAS THAT OF HUNGATE (1950). METHODS AND MEDIA FOR CULTURE ISOLATION AND STUDY OF PHYSIOLOGICAL CHARACTERISTICS WERE MAINLY THOSE OF BRYANT AND BURKEY (1953), BRYANT AND DOETSCH (1954), AND BRYANT AND SMALL (1956) WITH MINOR VARIATIONS IN THE COMPOSITION OF MEDIA AND IN THEIR PREPARATION.

ISOLATION MEDIUM BRYANT AND BURKEY (1953) AND BRYANT AND SMALL (1956) REPORTED THAT UNLIKE SIMILAR ORGANISMS FROM THE RUMEN, BUTYRIVIBRIO SPECIES GREW WELL IN GLUCOSE BROTH UNDER A NITROGEN ATMOSPHERE WITHOUT ADDED SODIUM BICARBONATE. THE ISOLATION TECHNIQUES USED IN THIS STUDY EMPLOYED THESE MODIFICATIONS IN AN ATTEMPT TO SELECT FOR MEMBERS OF THIS GENUS.

CULTURES WERE ISOLATED FROM RUMINAL INGESTA ON BRYANT AND BURKEY'S (1953) ISOLATION MEDIUM WHICH WAS MODIFIED TO CONTAIN 15 ML EACH OF THEIR MINERAL SOLUTIONS No. 1 AND No. 2, 0.4 GM OF GLUCOSE, 0.1 ML OF 0.1 PER CENT SOLUTION OF RESAZURIN AND 40 ML OF RUMEN FLUID PER 100 ML OF MEDIUM. THE PH WAS ADJUSTED TO 6.9 AND THE TOTAL VOLUME WAS BROUGHT UP TO 97.75 ML WITH WATER. EIGHT AND EIGHT-TENTHS ML OF THE MEDIUM WAS DISPENSED TO TUBES CONTAINING 0.18 GM AGAR. TWO TENTHS OF A ML OF FRESHLY PREPARED CYSTEINE HYDROCHLORIDE SOLUTION CONTAINING 0.1 GM OF CYSTEINE HYDROCHLORIDE IN 4.5 ML OF WATER

WAS THEN ADDED TO EACH TUBE AND THE TUBES WERE FLUSHED WITH NITROGEN. THE TUBES WERE THEN CLOSED WITH RUBBER STOPPERS WHICH WERE WIRED IN PLACE FOR STERILIZATION AT 121° C FOR 20 MIN. THE FINAL PH OF THIS MEDIUM WAS BETWEEN 6.8 AND 6.9. ALL MEDIA USED IN THIS STUDY CONTAINED CYSTEINE HYDROCHLORIDE AND WERE PREPARED BY THE SAME METHOD AS FOR THE ISOLATION MEDIUM.

THE RUMEN FLUID USED IN THESE MEDIA WAS PREPARED BY THE METHOD OF GILL AND KING (1958), BUT THE FLASKS WERE FLUSHED WITH CARBON DIOXIDE BEFORE STERILIZATION AND STORAGE IN THE REFRIGERATOR.

DILUTION SAMPLES OF RUMEN FLUID WERE DILUTED IN THE ANAEROBIC DILUTION SOLUTION OF BRYANT AND BURKEY (1953). HOWEVER, SODIUM CARBONATE WAS OMITTED, SODIUM HYDROXIDE WAS USED TO ADJUST THE PH, AND NITROGEN WAS BUBBLED THROUGH THE SOLUTION TO REPLACE ANY OXYGEN PRESENT.

SAMPLE TREATMENT, DILUTION SERIES, AND ROLL TUBE PREPARATION WERE ACCORDING TO THE METHODS DESCRIBED BY BRYANT AND BURKEY (1953) EXCEPT THAT NITROGEN WAS USED INSTEAD OF CARBON DIOXIDE TO MAINTAIN ANAEROBIC CONDITIONS. THE TUBES WERE INCUBATED AT 39° C FOR 3 DAYS.

ISOLATION ALL COLONIES FROM ROLL TUBE CULTURES INOCULATED WITH 1×10^{-8} DILUTIONS OF RUMEN SAMPLES WERE ISOLATED. PURE CULTURES WERE OBTAINED BY TWICE SERIALLY DILUTING THE WELL-ISOLATED COLONIES IN TUBES OF THE ISOLATION MEDIUM AND

PREPARING ROLL TUBE CULTURES. MACROSCOPIC AND MICROSCOPIC OBSERVATION OF SEVERAL COLONIES IN EACH TUBE VERIFIED THE PRESENCE OF SINGLE MORPHOTYPES. ISOLATED CULTURES WERE MAINTAINED ON THE RUMEN FLUID-GLUCOSE-CELLOBIOSE AGAR OF BRYANT AND BURKEY (1953).

CULTURAL TESTS MEDIA AND METHODS FOR STUDIES OF MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE PRESENT STRAINS WERE THOSE OF BRYANT AND DOETSCH (1954) AND BRYANT AND SMALL (1956).

FERMENTATION CHARACTERISTICS FERMENTATION CHARACTERISTICS WERE DETERMINED IN THE RUMEN FLUID GLUCOSE MEDIUM OF BRYANT AND SMALL (1956) CONTAINING 20 PER CENT RUMEN FLUID, MINERALS, 0.05 PER CENT CYSTEINE HYDROCHLORIDE, 0.4 PER CENT GLUCOSE, AND 0.2 PER CENT SODIUM BICARBONATE. THE MEDIUM WAS MODIFIED TO CONTAIN 0.0001 PER CENT RESAZURIN AND WAS ADJUSTED TO PH 6.9 BEFORE STERILIZATION UNDER A NITROGEN ATMOSPHERE. AFTER STERILIZATION THE PH OF THE MEDIUM WAS 7.0. IN ONE EXPERIMENT THE RUMEN FLUID OF THIS MEDIUM WAS REPLACED BY 1.5 PER CENT BBL TRYPTICASE AND 0.5 PER BACTO YEAST EXTRACT.

EFFECT OF PH AND PRODUCT CONCENTRATION FOR STUDIES OF THE EFFECT OF PH ON FATTY ACID PRODUCTION, 0.03 M K_2HPO_4 AND REQUIRED AMOUNTS OF CONC. H_3PO_4 WERE ADDED TO THE RUMEN FLUID-GLUCOSE MEDIUM. BEFORE STERILIZATION THE BUFFERED PH VALUES FOR THESE MEDIA WERE 7.0, 6.5, 6.0, AND 5.5. AFTER STERILIZATION THE PH VALUES WERE 7.07, 6.59, 6.10, AND 5.25 RESPECTIVELY.

ONE AND ELEVEN-HUNDREDTHS MILLIEQUIVALENTS OF REAGENT GRADE ACETIC, N-BUTYRIC, PROPIONIC, AND LACTIC ACIDS WERE ADDED PER 100 ML OF MEDIUM IN EXPERIMENTS TO DETERMINE THE EFFECT OF PRODUCT CONCENTRATION UPON FERMENTATION ACTIVITY. THE ACIDS WERE ADDED INDIVIDUALLY OR MIXED TO BUFFERED RUMEN FLUID MEDIUM. ALL OF THE MEDIA WERE ADJUSTED TO A PH OF 6.5 BEFORE STERILIZATION.

FERMENTATION IN RUMEN FLUID A RUMEN FLUID MEDIUM WAS PREPARED BY CENTRIFUGATION OF FRESH RUMEN FLUID AT 20,000 x G FOR 30 MIN. ONE-HALF OF ONE PER CENT CASEIN HYDROLYZATE, 1.5 PER CENT GLUCOSE, 0.05 PER CENT CYSTEINE HYDROCHLORIDE AND 0.0001 PER CENT RESAZURIN WERE ADDED TO THE RUMEN FLUID SUPERNATANT. THE PH OF THIS MEDIUM WAS ADJUSTED TO 6.9 BEFORE STERILIZATION. NITROGEN OR CARBON DIOXIDE WAS USED TO FLUSH THE MEDIUM.

ALL FERMENTATION TESTS WERE INOCULATED WITH ONE DROP OF 24 HR CULTURE GROWN IN RUMEN FLUID-GLUCOSE MEDIUM AND WERE THEN INCUBATED AT 37° C FOR 14 DAYS UNDER A NITROGEN ATMOSPHERE.

FOR IDENTIFICATION OF BUTYRIVIBRIO CULTURES, FATTY ACIDS WERE DETERMINED BY THE METHOD OF LANGSTON (1955). FOR OTHER FERMENTATION EXPERIMENTS THEY WERE DETERMINED BY THE METHOD OF SMITH, ET. AL. (1956).

RESULTS

DUPLICATE SAMPLES OF RUMEN INGESTA WERE TAKEN FROM THE TOP AND BOTTOM OF THE RUMEN OF A FISTULATED STEER FED A WINTER RATION OF POOR QUALITY ALFALFA HAY AND 4 LBS OF GRAIN CONCENTRATE PER DAY. FOURTY-NINE COLONIES DEVELOPED IN 8 ROLL TUBES INOCULATED WITH 1×10^{-8} DILUTIONS OF RUMEN FLUID. TEN OF THE COLONIES WERE FOUND TO CONTAIN ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, CURVED RODS WHICH PRODUCED PREDOMINATELY BUTYRIC ACID, AND USUALLY LESSER AMOUNTS OF FORMIC, LACTIC, PROPIONIC AND SUCCINIC ACIDS. STRAINS DESIGNATED A AND C WERE OBTAINED FROM 2 TOP SAMPLES TAKEN IN THE DORSAL SAC APPROXIMATELY 6" BELOW THE SURFACE OF THE HAY MAT, AND D FROM 1 BOTTOM SAMPLE TAKEN APPROXIMATELY 4" ABOVE THE FLOOR OF THE VENTRAL SAC OF THE RUMEN. STRAIN G WAS ISOLATED BY GILL AND KING (1958). THERE WAS CONSIDERABLE VARIATION IN THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS BETWEEN THE 11 ISOLATES STUDIED. THE MORPHOLOGICAL AND COLONIAL CHARACTERISTICS OF THE ISOLATES ARE GIVEN IN TABLE I.

THE ORGANISMS GREW WELL IN A RUMEN FLUID-GLUCOSE MEDIUM. TWENTY-FOUR HOUR CULTURES OF STRAINS, A4, C5, C7, C8, C9, C13, AND G SHOWED HEAVY TURBIDITY. STRAIN C15 HAD FLOCCULENT SEDIMENT. STRAINS D3, D5, AND D11 HAD GRANULAR SEDIMENTS AND ADHERED TO THE SIDES OF THE TUBES.

THE ORGANISM ALSO GREW WELL IN RUMEN FLUID-GLUCOSE MEDIUM WITH NITROGEN IN PLACE OF CARBON DIOXIDE AND WITH BICARBONATE OMITTED.

NO VISIBLE GROWTH OR PH REDUCTION WAS FOUND IN ANY CULTURE AT 50 C OR AT 22 C. THEY ALL GREW WELL AT 30 C, 37 C AND 39 C. VARIABLE GROWTH WAS OBTAINED AT 45 C.

NONE OF THE CULTURES PRODUCED ACETYL METHYL CARBINOL OR INDOL OR LIQUEFIED GELATIN. H₂S PRODUCTION, CASEIN AND STARCH DIGESTION AND NITRATE REDUCTION VARIED BETWEEN STRAINS AS SHOWN IN TABLE 2.

ALL OF THE CULTURES PRODUCED ACID FROM XYLOSE, GLUCOSE, FRUCTOSE, SUCROSE, LACTOSE, CELLOBIOSE, RAFFINOSE AND SALICIN, BUT NONE OF THEM FERMENTED SORBITOL OR MANNITOL. NO VISIBLE LOSS OF CELLULOSE WAS FOUND FROM TUBES OF RUMEN FLUID-CELLULOSE BROTH AFTER ONE WEEK OF INCUBATION.

THE ORGANISMS GREW WELL WHEN RUMEN FLUID WAS REPLACED BY YEAST EXTRACT AND TRYPTICASE IN THE GLUCOSE MEDIUM. ANALYSIS OF FATTY ACIDS SHOWED THAT WITHOUT RUMEN FLUID MUCH LARGER QUANTITIES OF BUTYRIC ACID WERE PRODUCED, AND THE ACETIC-FORMIC GROUP WAS INCREASED RATHER THAN DECREASED AS IN THE OTHER MEDIA. CONCENTRATIONS OF FATTY ACIDS PRODUCED BY THE 11 STRAINS IN RUMEN FLUID-GLUCOSE MEDIUM WERE DETERMINED BY THE METHOD OF LANGSTON (1955). RESULTS ARE SHOWN IN TABLE 3.

TABLE 4 SHOWS FATTY ACID PRODUCTION WHEN THE STRAINS WERE CULTURED IN RUMEN FLUID-GLUCOSE MEDIUM BUFFERED AT GRADED PH VALUES. NONE OF THE STRAINS GREW AT PH 5.25 EXCEPT STRAIN A4.

RESULTS OF ADDITION OF ACIDS TO THE BUFFERED MEDIUM ARE RECORDED IN TABLES 5, 6, 7 AND 8. THE ADDED FATTY ACIDS HAVE

ALREADY BEEN SUBTRACTED FROM THE RESULTS IN THESE TABLES. THE UNDERLINED FIGURES INDICATE THE FATTY ACID WHICH WAS ADDED IN EACH TREATMENT.

STRAINS A4, C7, C15 AND G WERE CULTURED IN THE 98 PER CENT RUMEN FLUID MEDIUM UNDER A NITROGEN ATMOSPHERE AND UNDER A CARBON DIOXIDE ATMOSPHERE. THE RESULTS OF FATTY ACID ANALYSES FROM THESE CULTURES ARE GIVEN IN TABLES 9 AND 10.

CULTURES OF FOUR STRAINS WERE SUBSAMPLED DAILY DURING 14-DAY INCUBATION PERIODS. ANALYSES OF THE FATTY ACID PRODUCTS INDICATED THAT THERE WAS NO CONSISTENT CHANGE IN THE RATIO OF FATTY ACIDS PRODUCED, AND THAT FATTY ACID PRODUCTION IN RUMEN FLUID-GLUCOSE MEDIUM WAS MOST RAPID DURING THE FIRST 24-HOUR PERIOD.

DISCUSSION

THE 10 STRAINS OF BUTYRIVIBRIO ISOLATED ON THE SEMI-SELECTIVE MEDIUM REPRESENTED ONE-FIFTH OF THE ORGANISMS CULTURED FROM 10^{-8} DILUTIONS OF RUMEN FLUID. COMPARED WITH THE TYPE CULTURE REPORTED BY BRYANT AND SMALL (1956), THESE STRAINS HAD IDENTICAL CHARACTERISTICS IN THAT THEY WERE ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, CURVED RODS PRODUCING CONSIDERABLE AMOUNTS OF BUTYRIC ACID. THEY ALSO FERMENTED XYLOSE, FRUCTOSE, GLUCOSE, CELLOBIOSE, SUCROSE, LACTOSE, AND SALICIN AND DID NOT FERMENT MANNITOL, INOSITOL, OR CELLULOSE (AFTER SEVERAL LABORATORY TRANSFERS). THE STRAINS VARIED IN

OTHER CHARACTERISTICS SUCH AS CASEIN DIGESTION, HYDROGEN SULFIDE PRODUCTION AND NITRATE REDUCTION. SIMILAR VARIATIONS ALSO OCCURRED AMONG STRAINS DESCRIBED BY BRYANT AND SMALL (1956). ACCORDING TO THE RESULTS SHOWN IN TABLES 9 AND 10, ALL TESTED STRAINS PRODUCED MORE FATTY ACID UNDER A CARBON DIOXIDE ATMOSPHERE THAN UNDER NITROGEN IN THE SAME MEDIUM. THIS MAY SIMPLY REFLECT THE HIGH REQUIREMENT FOR CARBON DIOXIDE REPORTED BY GILL AND KING (1958). THE SATISFACTORY GROWTH ON A MEDIUM FROM WHICH RUMEN FLUID WAS OMITTED MIGHT INDICATE THAT BUTYRIVIBRIO SPECIES ARE NOT AS FASTIDIOUS IN THEIR REQUIREMENTS AS SEVERAL OTHER RUMEN ORGANISMS REPORTED (BRYANT AND DOETSCH, 1955). IT WOULD APPEAR THAT A RANGE OF BUTYRIVIBRIO TYPES EXISTS IN THE RUMEN SINCE NO TWO STRAINS WERE IDENTICAL.

FERMENTATION CHARACTERISTICS OF ALL OF THE STRAINS INCLUDING THAT ISOLATED BY GILL AND KING (1958) WERE VERY SENSITIVE TO ENVIRONMENTAL CHANGES WHICH ARE KNOWN TO TAKE PLACE IN THE RUMEN (SMITH, ET. AL., 1956). FROM THE DATA IN TABLE 4 IT IS EVIDENT THAT THE RATIO OF FERMENTATION ACIDS WAS DEPENDENT UPON THE PH OF THE MEDIUM. GENERALLY THERE WAS A TENDENCY FOR CULTURES IN RELATIVELY ALKALINE MEDIA TO PRODUCE HIGHER AMOUNTS OF THE ACETIC (PLUS FORMIC) AND LACTIC (PLUS SUCCINIC) ACIDS THAN AT LOWER PH VALUES. BUTYRIC AND PROPIONIC ACIDS WERE FORMED IN HIGHER CONCENTRATIONS IN ACIDIC MEDIA.

THE ADDITION OF FATTY ACIDS TO THE MEDIUM AT CONCENTRATIONS WELL BELOW MAXIMUM PHYSIOLOGICAL RUMEN LEVELS MODIFIED THE RATIO OF FERMENTATION PRODUCTS. STRAINS A4 AND C7 WERE ABLE TO REDUCE LACTATE TO PROPIONATE. ALL 4 TESTED STRAINS APPARENTLY CONVERTED EITHER ACETATE OR PROPIONATE TO BUTYRATE. THIS ACTIVITY WAS EVIDENT IN BOTH THE RUMEN FLUID-GLUCOSE BROTH AND IN THE 98 PER CENT RUMEN FLUID MEDIUM. FATTY ACID PRODUCTION BY SOME OF THE STRAINS WAS INHIBITED BY INCREASED CONCENTRATIONS OF ADDED FERMENTATION FATTY ACIDS, HOWEVER, STRAIN G AND STRAIN C15 PRODUCED INCREASED AMOUNTS OF LACTIC ACID IN THE PRESENCE OF ADDED LACTIC ACID. THE PRODUCTION OF ACETIC ACID IN ITS ABSENCE FROM THE MEDIUM, AND ITS UTILIZATION IN ITS PRESENCE INDICATE THAT THIS GENUS IS PROBABLY NOT IMPORTANT IN ACETATE PRODUCTION IN THE RUMEN SINCE THE PRODUCTION MECHANISM IS SO SENSITIVE TO SUBSTRATE LEVELS.

THE SHIFT IN THE RATIO OF RUMEN FERMENTATION PRODUCTS WHEN RATIONS OF RUMINANTS ARE CHANGED (BRIGGS, ET. AL., 1957 AND REID, ET. AL., 1957) MAY BE CAUSED BY TWO MECHANISMS. AS DEMONSTRATED BY POUNDEN AND HIBBS (1948) AND PHILLIPSON (1952) THE TYPES OF RUMEN ORGANISMS MAY VARY WITH THE RATION OF THE ANIMAL. A SECOND MECHANISM IS SUGGESTED BY THE PRESENT DATA. THE SENSITIVITY OF THE METABOLISM OF BUTYRIVIBRIO TO ENVIRONMENTAL CHANGES, AND THE APPARENT INTERCONVERSION OF FERMENTATION PRODUCTS BY MEMBERS OF THIS GENUS DEMONSTRATE THAT INDIVIDUAL ORGANISMS WHICH OCCUR IN SIGNIFICANT NUMBERS

IN THE RUMEN ARE CAPABLE OF MAJOR CHANGES IN THEIR FERMENTATION. THESE CHANGES TAKE PLACE UNDER THE INFLUENCE OF ENVIRONMENTAL CHANGES WHICH ARE COMPATIBLE WITH PHYSIOLOGICAL CONDITIONS IN THE RUMEN.

SUMMARY

TEN STRAINS OF ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, BUTYRIC ACID-PRODUCING CURVED RODS HAVE BEEN ISOLATED FROM INGESTA OF THE BOVINE RUMEN. THESE 10 STRAINS OF BUTYRIVIBRIO REPRESENTED 1/5 OF ALL ISOLATES AT 1×10^{-8} DILUTIONS. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE 10 STRAINS AND A STRAIN ISOLATED BY GILL AND KING (1958) HAVE ALSO BEEN STUDIED. NO TWO OF THE ISOLATES WERE IDENTICAL IN ALL REACTIONS. MOST OF THE ORGANISMS PRODUCED A LARGE AMOUNT OF BUTYRIC AND SOME LACTIC, FORMIC, PROPIONIC AND SUCCINIC ACIDS WITH THE UTILIZATION OF ACETIC ACID IN A RUMEN FLUID GLUCOSE MEDIUM.

THE FERMENTATION CARRIED ON BY THESE ORGANISMS WAS SENSITIVE TO MOST TESTED ENVIRONMENTAL CHANGES. STUDIES WITH BUFFERED RUMEN FLUID-GLUCOSE MEDIA DEMONSTRATED A SHIFT OF THE FERMENTATION PRODUCTS WITH PH. ADDITION OF FATTY ACIDS TO THIS MEDIUM INDICATED THAT THESE ORGANISMS WERE ACTIVE IN THE CONVERSION OF ACETATE AND POSSIBLY PROPIONATE TO BUTYRATE. TWO STRAINS APPARENTLY HAD THE ABILITY TO PRODUCE PROPIONATE AT THE EXPENSE OF LACTATE. THE RESULTS OF THE FERMENTATION

TESTS IN 98 PER CENT RUMEN FLUID MEDIUM SHOWED THAT THE TESTED STRAINS USED ACETIC (PLUS FORMIC) OR LACTIC (PLUS SUCCINIC) TO PRODUCE BUTYRIC OR PROPIONIC ACID, AND PRODUCED HIGHER CONCENTRATIONS OF FATTY ACIDS UNDER A CARBON DIOXIDE ATMOSPHERE THAN UNDER NITROGEN. WHEN RUMEN FLUID AND ACETIC ACID WERE ABSENT ALL STRAINS HAD THE ABILITY TO PRODUCE EITHER FORMIC OR ACETIC ACID.

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TABLE I

MORPHOLOGICAL AND COLONIAL CHARACTERISTICS OF 11 STRAINS OF BUTYRIVIBRIO
GROWN ON RGCA SLANTS

STRAIN	SHAPE	CELLS (18 HR)		FLAGELLUM*		COLONIES (72 HR)	
		ARRANGEMENT	SIZE (μ)	WAVE LENGTH (μ)	AMPLITUDE (μ)	APPEARANCE	DIAMETER (MM)
A4	C.R.	SINGLE AND SHORT CHAINS	0.3-0.4 x 1.4-2.7	2.11	0.38	TRANSLUCENT LIGHT TAN	1.0
C5	S.C.R.	"	0.2-0.4 x 1.4-2.4	2.41	0.53	"	0.5
C7	V.S.C.R.	"	"	1.98	0.33	"	IRREG.
C8	"	"	0.3-0.5 x 2.0-2.7	2.44	0.41	"	0.5-1.0
C9	"	"	0.3-0.4 x 1.4-3.4	2.38	0.40	"	IRREG.
C13	"	"	0.2-0.3 x 1.7-2.4	1.98	0.36	"	1.0-2.0
C15	"	LONG CHAINS	0.6-0.7 x 2.7-5.4	1.98	0.33	LIGHT TAN	1.0-2.0
D3	"	SINGLE, SHORT AND LONG CHAINS	0.6-0.7 x 2.4-4.0	2.64	0.61	FLLAMENTOUS TAN	1.0
D5	"	SHORT AND LONG CHAINS	0.3-0.4 x 2.0-2.7	1.98	0.33	"	1.0-1.5
D11	"	LONG CHAINS	0.3-0.5 x 5.7-6.7	1.65	0.26	"	2.0-2.5
G	S.C.R.	SINGLE AND SHORT CHAINS	0.3-0.4 x 2.4-3.0	1.91	0.33	TRANSLUCENT LIGHT TAN	IRREG.

C = CURVED, R = RODS, S = SLIGHT, V = VERY

*GLUCOSE CONCENTRATION OF THE MEDIUM REDUCED TO 0.1 PER CENT

TABLE 2
SOME VARIABLE CHARACTERISTICS AMONG 11 STRAINS OF
BUTYRIVIBRIO

	STRAIN										
	A4	C5	C7	C8	C9	C13	C15	D3	D5	D11	G
H ₂ S PRODUCTION	SL.	-	+	-	-	-	-	-	-	-	-
CASEIN DIGESTION	-	-	-	-	-	-	-	-	-	-	+
NITRATE REDUCTION	+	-	-	-	-	-	-	-	-	-	-
STARCH HYDROLYSIS	+	-	-	-	-	-	+	+	+	+	+
FINAL PH OF MEDIUM	5.07	5.30	5.25	5.25	5.20	5.30	5.25	5.16	5.13	5.14	5.40

SL. = SLIGHT

TABLE 3

FATTY ACID PRODUCTION IN RUMEN FLUID GLUCOSE MEDIUM BY STRAINS OF
BUTYRIVIBRIO DETERMINED BY THE METHOD OF LANGSTON (1955)
(14 DAY CULTURES)

STRAIN	ACID PRODUCED (MEQ/100 ML)						TOTAL
	BUTYRIC	PROPIONIC	ACETIC	FORMIC	SUCCINIC	LACTIC	
A4	1.11	0.73	-0.84	0.64	0.05	0.05	1.74
C5	0.32	-0.09	-1.42	0.31	0.00	0.30	-0.58
C7	1.52	1.05	-0.82	0.85	0.15	-0.26	2.49
C8	0.32	-0.35	-1.43	0.71	0.00	0.10	-0.65
C9	1.21	-0.10	-1.50	0.10	0.02	0.89	0.62
C13	0.59	-0.25	-0.94	0.23	0.02	0.70	0.35
C15	0.74	0.04	-0.92	0.77	0.17	0.63	1.43
D3	0.59	-0.12	-0.46	0.61	0.00	0.28	0.90
D5	0.85	-0.25	-0.90	0.56	0.00	0.08	0.34
D11	1.44	-0.24	-1.05	0.78	0.04	0.01	0.98
G	0.39	0.12	-0.74	0.18	0.00	1.48	1.43
AVERAGE	0.83	0.05	-1.00	0.52	0.04	0.38	0.82
BLANK VALUES							
FOR MEDIUM	0.95	0.98	2.13	0.75	0.21	0.82	

TABLE 4

FATTY ACIDS PRODUCED IN BUFFERED RUMEN FLUID GLUCOSE
MEDIA BY 11 STRAINS OF BUTYRIVIBRIO
(14 DAY CULTURE)

STRAIN	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	7.07	0.60	0.95	0.65	1.41
	6.59	0.95	0.98	0.57	0.55
	6.10	1.36	1.12	-0.37	0.18
	5.25	0.18	0.10	-0.36	0.10
C5	7.07	0.52	-0.02	0.17	2.72
	6.59	0.60	-0.01	0.08	1.83
	6.10	0.49	0.03	-0.19	1.38
C7	7.07	0.43	1.34	0.74	1.53
	6.59	1.13	0.87	0.01	0.53
	6.10	1.80	1.19	-0.54	0.13
C8	7.07	0.52	-0.03	0.09	2.52
	6.59	0.72	-0.02	-0.42	3.01
	6.10	0.38	0.10	-0.11	1.25
C9	7.07	0.60	-0.04	-0.22	1.98
	6.59	1.39	0.06	-0.82	2.98
	6.10	0.80	0.08	-0.73	1.59
C13	7.07	0.54	0.01	0.18	2.46
	6.59	0.78	0.02	-0.21	2.26
	6.10	0.58	0.02	-0.24	1.78
C15	7.07	1.00	0.10	0.39	3.22
	6.59	1.69	0.12	0.05	1.80
	6.10	1.52	0.08	-0.44	1.08
D3	7.07	1.16	0.01	0.40	1.88
	6.59	1.70	0.07	0.15	1.65
	6.10	1.23	-0.02	-0.35	1.35
D5	7.07	1.42	-0.01	0.43	1.41
	6.59	1.52	0.13	-0.58	1.13
	6.10	1.41	-0.02	-0.22	1.04
D11	7.07	1.02	-0.04	0.37	1.92
	6.59	1.79	-0.07	0.36	1.51
	6.10	1.22	0.09	-0.40	1.15
G	7.07	0.39	0.43	0.07	2.16
	6.59	0.71	0.13	-0.15	2.36
	6.10	0.70	0.14	-0.50	1.28

TABLE 5

FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS^x IN
A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN A4
(14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>1.20</u>	0.85	0.33	0.78
2	6.56	1.76	<u>0.25</u>	0.57	0.68
3	6.55	1.29	0.81	<u>0.19</u>	0.97
4	6.55	1.33	1.40	0.63	<u>-0.03</u>
5	6.35	<u>1.37</u>	<u>1.14</u>	<u>0.46</u>	<u>0.04</u>
CONTROL	6.35	1.23	0.90	0.21	0.75

^xTHE UNDERLINED VALUE INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEQ/100 ML IN EACH TREATMENT.

TABLE 6

FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS^x IN
A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN C7
(14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEq/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>1.29</u>	0.79	0.00	1.13
2	6.56	1.74	<u>1.00</u>	-0.39	0.38
3	6.55	1.52	1.18	<u>-0.45</u>	0.23
4	6.55	1.18	1.56	0.03	<u>-0.39</u>
5	6.35	<u>1.52</u>	<u>1.21</u>	<u>0.22</u>	<u>-0.33</u>
CONTROL	6.35	1.34	0.96	-0.12	0.67

^xTHE UNDERLINED VALUE INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEq/100 ML IN EACH TREATMENT.

TABLE 7

FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS^x IN
A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN G
(14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>0.79</u>	0.02	-0.52	2.99
2	6.56	1.47	<u>0.12</u>	-0.44	1.75
3	6.55	1.21	0.02	<u>-0.65</u>	1.79
4	6.55	1.17	0.01	-0.62	<u>2.55</u>
5	6.35	<u>0.40</u>	<u>0.06</u>	<u>-0.45</u>	<u>2.76</u>
CONTROL	6.35	0.76	0.08	-0.93	1.70

^xTHE UNDERLINED VALUE INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEQ/100 ML IN EACH TREATMENT.

TABLE 8

FATTY ACIDS PRODUCED ON THE ADDITION OF FREE ACIDS^x IN
A BUFFERED RUMEN FLUID GLUCOSE MEDIUM BY STRAIN C15
(14 DAY CULTURE)

TREATMENT	BUFFERED PH	ACID PRODUCED (MEQ/100 ML)			
		<u>BUTYRIC</u>	<u>PROPIONIC</u>	<u>ACETIC PLUS FORMIC</u>	<u>SUCCINIC PLUS LACTIC</u>
1	6.54	<u>1.46</u>	0.07	0.09	2.19
2	6.56	1.78	<u>-0.15</u>	0.06	1.41
3	6.55	1.91	0.02	<u>-0.77</u>	1.61
4	6.55	1.71	-0.04	-0.14	<u>2.80</u>
5	6.35	<u>1.96</u>	<u>0.06</u>	<u>-0.62</u>	<u>2.24</u>
CONTROL	6.35	1.55	0.02	-0.34	1.44

^xTHE UNDERLINED VALUE INDICATES THE ACID ADDED AT THE RATE OF 1.11 MEQ/100 ML IN EACH TREATMENT.

TABLE 9

FATTY ACIDS PRODUCED IN A 98 PER CENT RUMEN FLUID MEDIUM
BY STRAINS OF BUTYRIVIBRIO UNDER NITROGEN ATMOSPHERE
(14 DAY CULTURE)

STRAIN	ACID PRODUCED (MEq/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	1.77	0.39	-1.05	0.77
C7	4.65	1.22	-4.15	-1.08
C15	3.44	0.57	-2.60	0.73
G	2.19	0.00	-1.12	1.46

TABLE 10

FATTY ACIDS PRODUCED IN A 98 PER CENT RUMEN FLUID MEDIUM
BY STRAINS OF BUTYRIVIBRIO UNDER CARBON DIOXIDE ATMOSPHERE
(14 DAY CULTURE)

STRAIN	ACID PRODUCED (MEq/100 ML)			
	BUTYRIC	PROPIONIC	ACETIC PLUS FORMIC	SUCCINIC PLUS LACTIC
A4	3.38	0.15	-2.15	1.26
C7	5.40	1.38	-4.06	-0.88
C15	3.51	0.56	-1.11	2.41
G	3.32	0.32	-2.51	0.89

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APPENDIX 2

1. COMPOSITION OF MODIFIED BRYANT AND BURKEY'S (1953A)

ISOLATION MEDIUM.

MINERAL SOLUTION No. 1

0.3% K_2HPO_4	15 ML.
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MINERAL SOLUTION No. 2

0.3% KH_2PO_4	
0.6% $(NH_4)_2SO_4$	
0.6% $NaCl$	15 ML.
0.06% $CaCl_2$	
0.06% $MgSO_4$	

RUMEN FLUID	40 ML.
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GLUCOSE	0.4 GM.
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0.1% RESAZURIN	0.1 ML.
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THE ABOVE MEDIUM WAS ADJUSTED WITH $NaOH$ TO PH 6.9 AND MADE UP TO 97.75 ML. WITH DISTILLED WATER. EIGHT AND EIGHT-TENTHS ML. OF THE MEDIUM WAS DISPENSED TO TUBES CONTAINING 0.18 GM. AGAR. TWO-TENTHS OF A ML. OF FRESHLY PREPARED CYSTEINE HYDROCHLORIDE SOLUTION CONTAINING 0.1 GM. OF CYSTEINE HYDROCHLORIDE IN 4.5 ML. OF WATER WAS THEN ADDED TO EACH TUBE AND THE TUBES WERE FLUSHED WITH NITROGEN. STOPPERED TUBES WERE STERILIZED AT 121 C FOR 20 MIN.

2. COMPOSITION OF MODIFIED ANAEROBIC DILUTION SOLUTION OF BRYANT AND BURKEY (1953A).

MINERAL SOLUTION No. 1 AND No. 2 (SAME AS ABOVE)	15 ML. EACH
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CYSTEINE HYDROCHLORIDE	0.05 gm.
0.1% RESAZURIN	0.1 ml.

THE SOLUTION WAS ADJUSTED WITH NaOH TO PH 6.9 AND MADE UP TO 100 ML. WITH DISTILLED WATER. AFTER STERILIZATION AT 121 C FOR 20 MIN., THE BOTTLES WERE BUBBLED WITH STERILE NITROGEN AND STOPPERED.

3. COMPOSITION OF THE MODIFIED TRYPTICASE AND YEAST EXTRACT MEDIUM (BRYANT AND SMALL, 1956A).

MINERAL SOLUTION No. 1 AND No. 2 (SAME AS ABOVE)	15 ML. EACH
TRYPTICASE	1.5 gm.
YEAST EXTRACT	0.5 gm.
GLUCOSE	0.4 gm.
NAHCO ₃	0.2 gm.
0.1% RESAZURIN	0.1 ml.

THIS MEDIUM WAS ADJUSTED WITH H₂SO₄ TO PH 6.9 AND MADE UP TO 97.75 ML. THE SAME QUANTITIES OF CYSTEINE HYDROCHLORIDE WERE ADDED TO THE TUBES AS FOR THE ISOLATION MEDIUM.

4. COMPOSITION OF THE MODIFIED RUMEN FLUID GLUCOSE MEDIUM OF BRYANT AND SMALL (1956A).

THE COMPOSITION AND PREPARATION OF THIS MEDIUM WERE SIMILAR TO THOSE FOR MEDIUM 3 MENTIONED ABOVE, EXCEPT THE YEAST EXTRACT AND TRYPTICASE WERE REPLACED BY 20 ML. OF RUMEN FLUID PER 100 ML. OF MEDIUM.

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ABSTRACT

TEN STRAINS OF ANAEROBIC, GRAM NEGATIVE, MONOTRICHOUS, BUTYRIC ACID-PRODUCING CURVED RODS HAVE BEEN ISOLATED FROM INGESTA OF THE BOVINE RUMEN. THESE 10 STRAINS OF BUTYRIVIBRIO REPRESENTED 1/5 OF ALL ISOLATES AT 1×10^{-8} DILUTIONS. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE 10 STRAINS AND A STRAIN ISOLATED BY GILL AND KING (1958) HAVE ALSO BEEN STUDIED. NO TWO OF THE ISOLATES WERE IDENTICAL IN ALL REACTIONS. MOST OF THE ORGANISMS PRODUCED A LARGE AMOUNT OF BUTYRIC AND SOME LACTIC, FORMIC, PROPIONIC AND SUCCINIC ACIDS WITH THE UTILIZATION OF ACETIC ACID IN A RUMEN FLUID GLUCOSE MEDIUM.

THE FERMENTATION CARRIED ON BY THESE ORGANISMS WAS SENSITIVE TO MOST TESTED ENVIRONMENTAL CHANGES. STUDIES WITH BUFFERED RUMEN FLUID-GLUCOSE MEDIA DEMONSTRATED A SHIFT OF THE FERMENTATION PRODUCTS WITH PH. ADDITION OF FATTY ACIDS TO THIS MEDIUM INDICATED THAT THESE ORGANISMS WERE ACTIVE IN THE CONVERSION OF ACETATE AND POSSIBLY PROPIONATE TO BUTYRATE. TWO STRAINS APPARENTLY HAD THE ABILITY TO PRODUCE PROPIONATE AT THE EXPENSE OF LACTATE. THE RESULTS OF THE FERMENTATION TESTS IN 98 PER CENT RUMEN FLUID MEDIUM SHOWED THAT THE TESTED STRAINS USED ACETIC (PLUS FORMIC) OR LACTIC (PLUS SUCCINIC) TO PRODUCE BUTYRIC OR PROPIONIC ACID, AND PRODUCED HIGHER CONCENTRATIONS OF FATTY ACIDS UNDER A CARBON DIOXIDE ATMOSPHERE THAN UNDER NITROGEN. WHEN RUMEN FLUID

AND ACETIC ACID WERE ABSENT ALL STRAINS HAD THE ABILITY TO
PRODUCE EITHER FORMIC OR ACETIC ACID.