THE SEPARATION AND MICRO-QUANTITATIVE DETERMINATION OF VOLATILE FATTY ACIDS IN RUMEN LIQUOR BY GAS-LIQUID PARTITION CHROMATOGRAPHY

By

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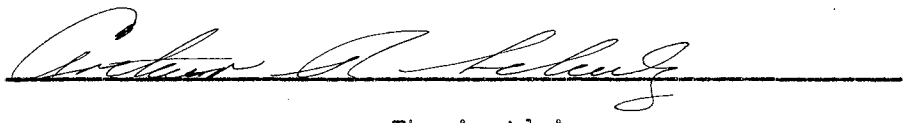

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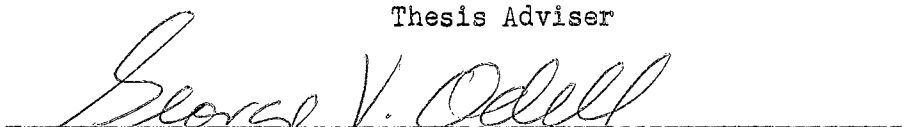

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CHAPTER I

INTRODUCTION

Probably no field of ruminant nutrition has received more emphasis in the past few years than the metabolism of the volatile fatty acids by the ruminant animal. It has, of course, been obvious that herbivora are capable of utilizing larger quantities of roughages than are carnivora. Knowledge of the anatomical differences in the digestive tract which permits classification of herbivora on the basis of whether or not they possess a rumen dates to the eighteenth century. Tappeiner (1) is credited with the first observation that the fermentation of cellulose in the rumen is associated with the formation of volatile fatty acids. He recognized that although several volatile fatty acids were found, acetic acid was the chief constituent.

For many years, it was believed that the volatile fatty acids formed in the rumen were of little nutritional significance. It has been realized only in the past thirty years that the product of bacterial fermentation of polysaccharides in the rumen is largely volatile fatty acids and not glucose (2-11). Consequently, a major physiological fuel for the ruminant is volatile fatty acids (7-16) and not glucose as is the case in non-ruminants. This is dramatically portrayed when one compares the blood levels of glucose, ketone bodies and volatile fatty acids of human blood with those which can be tolerated by the ruminant without ill effects. In Table I are shown the normal limits for human blood in milligrams per 100 milli-

TABLE I

GLUCOSE, KETONE BODIES, AND VOLATILE FATTY ACIDS

IN HUMAN BLOOD AND SHEEP BLOOD

	Human mg/100 ml blood	Sheep mg/100 ml blood
Glucose	50-90	18
Ketone bodies	0.2-0.8	12
Volatile fatty acids	2.05-5.26*	100

* This is the value given for the sum of the concentrations of acetic acid and formic acid; only minute amounts of other volatile fatty acids are present in human blood.

liter of blood (17) and the levels in sheep blood which have not produced ill effects (14).

This aspect of ruminant nutrition might be studied by two different approaches. One might study the production of volatile fatty acids, or one might investigate the utilization of volatile fatty acids by ruminant tissues. Evidence has been obtained which suggests that the molar ratio of the different volatile fatty acids produced can vary widely depending upon the ration fed (18-23). Artificial rumen techniques have been extensively employed for the purpose of studying, in a more closely controlled manner, the action of rumen microflora on various substrates (24 - 30). In a limited number of these investigations, the end products have been measured (31-34), but in the great majority of cases only substrate (usually cellulose) disappearance has been studied (26-30). If the molar ratio of volatile fatty acids produced is indeed a factor which can be varied, then estimation of substrate disappearance is certainly an unsatisfactory criterion.

The utilization of volatile fatty acids by ruminant tissues has been studied, but the conclusions drawn from these experiments are widely divergent (32-34). Even a cursory view of this field of research indicates the necessity for development of a suitable quantitative estimation of the individual volatile fatty acids on a small sample of rumen fluid, blood and body tissues. Such a procedure requires methods for the specific determination of the various fatty acids, or the separation of the individual acids followed by the quantitative determination of each of them. Enzymic procedures exist for the estimation of acetic acid (35) and propionic acid (36), but specific methods for butyric, iso-butyric, valeric and iso-valeric acids do not exist. For this reason,

a method was sought for the resolution of mixtures of volatile fatty acids. The individual short-chain fatty acids differ with respect to physical properties, while their chemical properties are quite similar. Therefore, methods developed for the resolution of mixtures of the short-chain acids are based on differences in physical properties.

Steam distillation has been employed for the separation of volatile fatty acids (8), but this method is more applicable for the separation of the total volatile fatty acids from non-volatile acids than for the resolution of mixtures of volatile fatty acids. Furthermore, steam distillation does not readily lend itself to micro-procedures. Behrens (37) and Werkman (38) introduced partition methods which depend on the fact that each fatty acid can be partitioned between water and some immiscible organic solvent. However, the reported methods appear to be of little use because they do not adequately resolve mixtures containing more than two acids. Indeed, any method which entails the partitioning of volatile fatty acids between water and an immiscible organic solvent is disadvantageous because of the great solubility of the short-chain fatty acids in water. Craig (39) has pointed out that identification by countercurrent distribution of a compound with a partition coefficient greater than 10 or less than 0.3 is unreliable.

A procedure based on fractional distillation was reported by Schicktanz, et al. (40). In this fractional distillation, the volatile fatty acids were actually separated, but the accurate quantitative evaluation of the individual acids was only an approximation because of azeotrope formation (40).

Smith (41) and also Marvel and Rands (42) demonstrated the use of silica gel partition chromatography for the separation of the mixture

of volatile fatty acids. This was applied to the biological material by Elsden (43). This method is not well suited to the study of ruminology for a variety of reasons. Perhaps the most important aspect which renders the procedure infeasible is the fact that the usual large-animal research involves extensive statistical analysis. Under these conditions, the cost of silicic acid, solvent, and labor becomes exorbitant. Chromatography on silica gel does not resolve butyric and higher fatty acids (43).

The development of gas-liquid partition chromatography by James and Martin (44) has provided tremendous impetus to the general field of chemical analysis. This method is sensitive, rapid and simple. In suitable cases the gas-liquid partition chromatography has several advantages over the ordinary liquid-liquid partition chromatography: ;) Owing to the low viscosity of the gas, it is possible to work with long columns, which yield high efficiencies. In the liquid-liquid partition chromatography, the length of column is usually about 40 cm (43), whereas the column employed in gas-liquid partition chromatography may be in the range of 4 to 11 feet in length (45). 2) Because of low gas viscosity, the column may be operated at high gas flow rates, so that analyses may be conducted rapidly in gas-liquid partition chromatography. The elution of volatile fatty acids through acetic acid from a silica gel column requires several hours time and large volumes of eluent (43). As will be shown in the text, a mixture containing volatile fatty acids from formic through valeric acid can be resolved by gas-liquid chromatography in twelve minutes. 3) The gas molecules are in general much smaller and less strongly adsorbed than the molecules of the vapor, so that the distribution of the vapor between the fixed and mobile phases is largely independent of the 'solvent' gas (46).

4) There are many convenient methods available for the detection of small vapor concentration in a gas such as making use of thermal conductivity (47), vapor-density-balance (48), photometric titration (44), hydrogen flame (49). Most of these are rapid in response and can readily be adapted to continuous automatic recording. It was reported (45) that gas-liquid partition chromatography has been used to separate complex mixtures of substances in amounts ranging from 10^{-15} to 70 grams, or volumes introduced can vary from 1 to 100 µl for liquids and up to 20 ml (at normal temperature and pressure) for gases on a 4-6 mm diameter analytical column. In liquid-liquid partition chromatography, 5-80 mg of acids mixture are required for analysis, and 0.5 mg of one acid could be detected in an 80 mg mixture under optimal conditions (42). A big advantage to gas-liquid chromatography is its high resolving power (50).

The system reported by James and Martin (44) separated the volatile fatty acids with a reasonably high degree of resolution. The degree of resolution has been improved by a more recent procedure (51). There are still many obstacles to the satisfactory use of gas-liquid partition chromatography for the analysis of volatile fatty acids in biological materials. An attempt was made to develop a procedure for the resolution and micro quantitative estimation of a total of approximately five microliters of mixture of volatile fatty acids in one or two milliliters of aqueous solution (rumen liquor). A number of the difficulties which were encountered were studied and resolved in the course of this investigation.

CHAPTER II

METHODS AND RESULTS

A. Comparison of Gas-Liquid Chromatography of Free Volatile Fatty Acids and Methyl Esters of Fatty Acids .

Volatile fatty acids may be separated by gas-liquid chromatography either as the free acids or as the methyl esters of the acids. Gas-liquid chromatography of long-chain fatty acids is greatly facilitated by the conversion of these acids to their respective methyl esters (52). The base-catalyzed formation of ethyl or methyl esters by transesterification using tri-ethyl phosphate or tri-methyl phosphate requires refluxing for two hours (53). The esterification of short-chain fatty acids by methanol can also be conducted as a base-catalyzed procedure (52), but reference to Table II shows that separation of the product esters from the methanol on the basis of differences in boiling-point or freezing-point temperatures is difficult. Methods for esterification which involve fractional distillation, such as the method of Weissberger and Kibler (55) require elaborate equipment and considerable manipulation. Numerous acid-catalyzed esterification procedures have been employed (56-58). These procedures are generally rapid and result in good yields. However, in order to satisfy the requirements for the esterification, the free acids must be obtained in an essentially anhydrous medium. When this requirement has been met, there is little advantage to esterification, as the free volatile fatty acids can be readily separated by gas-liquid chromatography (44, 51). Furthermore, the separation of the

TABLE II

BOILING POINTS AND FREEZING POINTS OF VOLATILE FATTY ACIDS AND THEIR METHYL ESTERS (54).

	Boiling Point		Freezing Point	
	Free acid °C.	Methyl ester °C.	Free acid °C.	Methyl ester °C.
Formic Acid	100.8	31.8	8.40	-99.0
Acetic Acid	118.1	57.3	16.6	-98.7
Propionic Acid	141.4	79.7	-20.8	-87.5
Iso-butyric Acid	154.7	92.3	-47	-87.7
n-Butyric Acid	164.1	102.8	- 5.5	-84.8
Iso-valeric Acid	176.5	116-7	-29.3	----
n-Valeric Acid	186.4	127.3	-34.5(-59)	-91

Methanol: b.p. 64.7° C., f.p. -97.8° C.
Gaseous HCl: b.p. -85° C., f.p. -111° C.

methyl esters of the volatile fatty acids from HCl and methanol is difficult. For these reasons, a satisfactory procedure was sought for the gas-liquid chromatography of the free volatile fatty acids.

B. Selection of a Method for Gas-Liquid Chromatography of Free Volatile Fatty Acids.

Raupp (51) has recently reported a procedure for the gas-liquid chromatography of volatile fatty acids which appears to have advantages over the system used by James and Martin (44). The elution pattern of the volatile fatty acids is more symmetrical in the case of the system developed by Raupp, and the separation of formic and acetic acids is sharper. As a result of the improved separation, the column temperature, and rate of gas flow can be increased, and the retention time is decreased.

1. Preparation of the Inert Supporting Medium.

Celite 545 was used as the inert supporting medium. It was size-graded by repeated suspension in water in a two liter graduated cylinder. The fine particles which did not settle in 3 minutes were discarded. The resultant celite was further purified according to the method of Lipsky (59). The procedure employed was as follows: The celite was suspended in concentrated hydrochloric acid and was allowed to stand for 15 minutes with occasional stirring. The slurry was then washed several times with water to remove the acid. An aqueous solution containing 5% KOH (w/w) was added to the celite and the slurry was allowed to stand for 10 minutes with intermittent stirring. The alkali was decanted, and the celite was suspended in distilled water and washed by filtration until the filtrate was neutral. The celite was spread on aluminum foil and left in an oven at 100° C. overnight. The celite was further size-graded by passing it

through screens of various sizes. The 40-60 size was retained and used in all of the experiments.

2. Preparation of Column Material.

The stationary phase developed by Raupp (51) consisted of dioctyl sebacate and sebacic acid. The sebacic acid was added to prevent dimerization of volatile fatty acids. The column material was prepared as follows: 8.5 grams of dioctyl sebacate and 1.5 grams of sebacic acid were dissolved in 60 milliliters of a 1:3 mixture (v/v) of ethyl ether:ethyl alcohol. This solution was then mixed with 30 grams of 40-60 mesh celite in an evaporating dish which was heated on a steam bath. The mixture was stirred continuously to maintain a uniform mixture. When the odor of alcohol and ether could no longer be detected, the celite containing dioctyl sebacate and sebacic acid was spread out on a sheet of aluminum foil, and placed in an oven at 150° C. for overnight. During this heating period, a volatile contaminant was removed which would otherwise clog the column with a waxy material after the vapors emerged from the postheater. The column material prepared in this manner was packed into a coiled glass chromatograph column with an internal diameter of 4 mm. and a length of approximately 11 feet. The column material was added slowly in small portions from one end of the column. A slight vacuum from a water aspirator was applied to the other end of the glass column. The uniform packing of the column was facilitated by vibrating the column with a small electric vibrator. A certain degree of skill is required to obtain a firm, uniform packing of the column.

3. Gas-Liquid Chromatography of the Mixtures of Volatile

Fatty Acids.

The gas-liquid chromatography apparatus used in these experiments was constructed and donated by the Continental Oil Company, Ponca City, Oklahoma. This instrument employed a thermal conductivity detector (47). Helium gas was used as the carrier gas. Accurate control of the rate of flow of the carrier gas was achieved through two sets of pressure regulators mounted in series. The gas flow-rate was measured at the column outlet with a standard gas flow burette and stop-watch. The flow-rate was corrected for temperature and pressure.

The logarithm of the retention volumes of the acids versus the number of carbon atoms are plotted in Fig. 1. The retention volume is defined as the volume of mobile phase required to elute the maximum of a peak. (45). Straight-line relationships were obtained for the normal acids from acetic to valeric, and also for the branched-chain acids. The slopes of the lines for the normal acids and the iso-acids appear to be identical, but the intercepts differ. The logarithm of the retention volumes for formic acid deviates from the line of the other normal acids. This deviation was also noted by James and Martin (44). A plot of the logarithm of the retention volumes against carbon number is a valuable aid to the qualitative identification of an unknown acid (61).

Fig. 2 shows the linear relationship between the logarithms of the retention volumes and the reciprocals of absolute temperatures for the various volatile acids tested. The slope of these lines would be expected to be proportional to the heat of vaporization. Furthermore, since these values are a characteristic of the components in a particular liquid

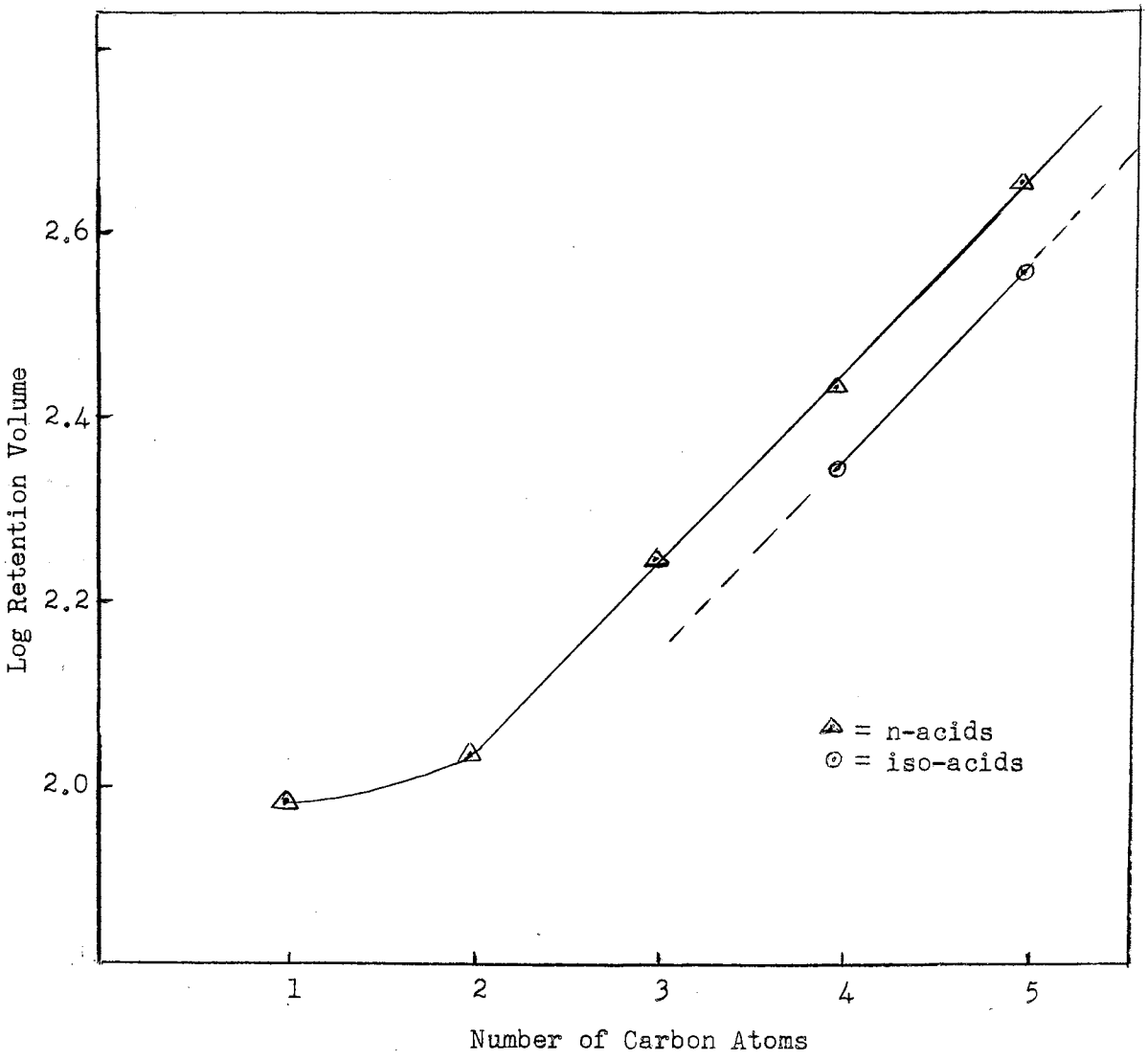

Fig. 1. Logarithm of the Retention Volumes Plotted Against Number of Carbon Atoms. Column materials: (1) Inert support: Kieselguhr (celite 545) 40-60 mesh; (2) Liquid phase: 15% sebacic acid in dioctyl sebacate. Column temperature: 150° C.; Preheater temperature: 264° C.; Cell temperature: 258° C.; Carrier gas: helium gas; Gas flow rate: 60.6 ml/min.; Attenuation: 15.

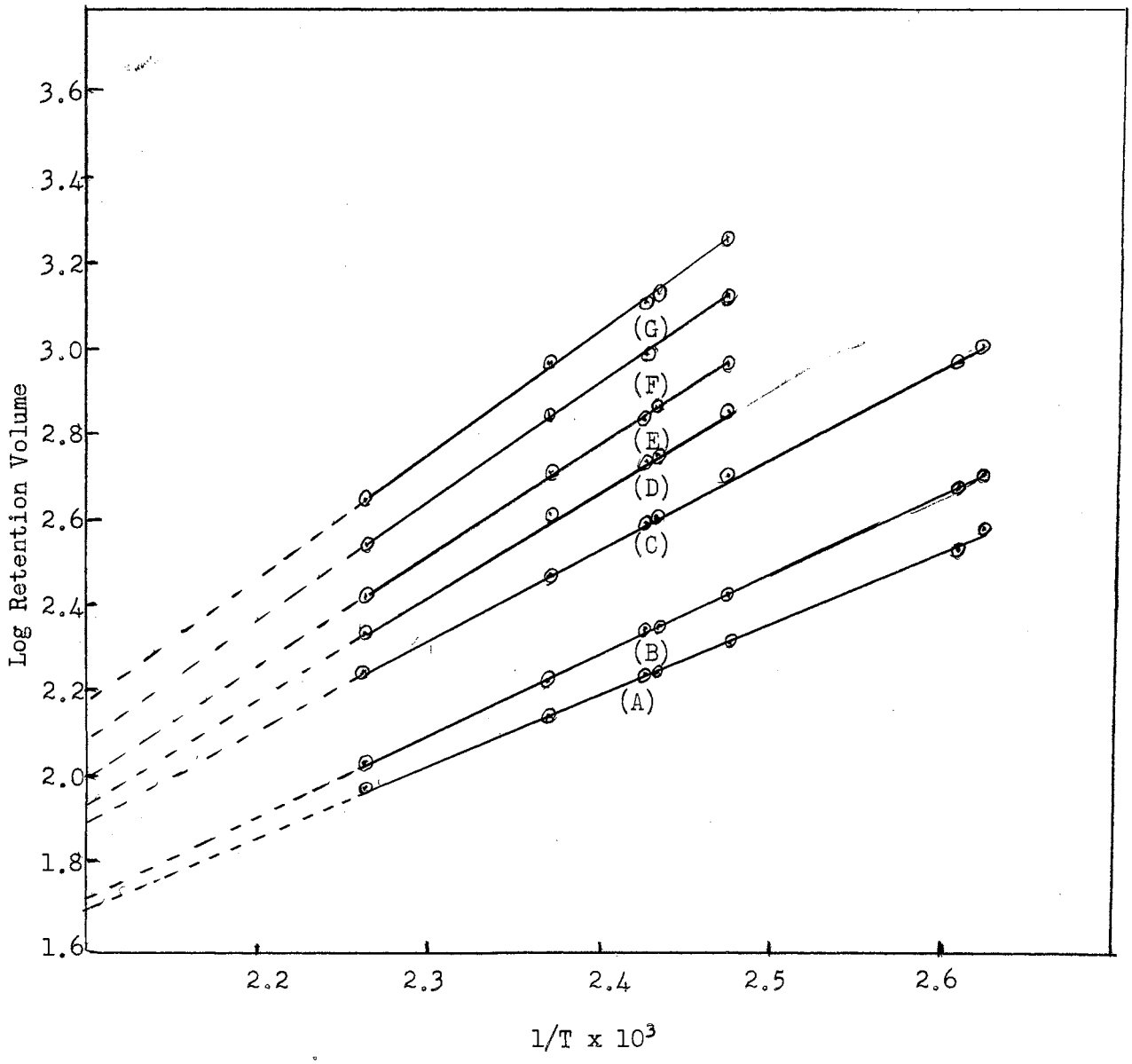

Fig. 2. Logarithm of the Retention Volumes Plotted Against Reciprocal Absolute Temperatures. (A) Formic acid; (B) Acetic Acid; (C) Propionic acid; (D) Iso-butyric acid; (E) n-Butyric acid; (F) Iso-valeric acid; (G) n-Valeric acid; Inert support: Kieselguhr (celite 545), 40-60 mesh; Liquid phase: 15% sebacic acid in dioctyl sebacate; Carrier gas: helium.

Column Temperature in absolute degrees	Gas Flow Rate ml/min.	Column Temperature in absolute degrees	Gas Flow Rate ml/min.
381	69.8	410	61.8
383	69.8	422	60.6
388	69.0	442	54.54
404	65.2		

phase, these data are useful for comparison with other liquid phases which might be employed in the future.

C. Preparation of Moisture-Free Sodium Salts of the Volatile Fatty Acids.

Before fluids such as rumen liquor can be analyzed for their volatile fatty acids content by gas-liquid chromatography, the volatile fatty acids must be concentrated. There are several considerations which render this a necessary operation. Porter, et al. (60) have demonstrated that maximum column efficiency can be obtained only when the volume of sample injected in the column is small. Instantaneous introduction to the column is a requisite to sharp separation of the components of the mixture, and this requirement can be met only when the injected sample is sufficiently small to undergo instantaneous vaporization. Furthermore, the detector employed will detect any gas or vapor having a thermal conductivity differing from that of helium. A diluting material, such as water, will elicit a response. The concentration of water in rumen liquor is so much greater than that of fatty acids that the large water elution peak would completely obscure the response due to the presence of volatile fatty acids. Accordingly, two ml. of the solution containing the volatile fatty acids were transferred to micro-lyophilization tubes which contained a 28/15 ground-glass ball joint. The solution was adjusted to pH 10 with 1 N NaOH. As many as 12 of these tubes, each containing sodium salts of volatile fatty acids can be placed on the lyophilization apparatus at one time. The water was thus removed by lyophilization and the tubes containing the moisture-free sodium salts were stored in a desicator.

D. Conversion of Sodium Salts to the Corresponding Free Volatile Acids and their Transfer to the Column.

Prior to gas-liquid chromatography, sodium salts must be converted to free volatile fatty acids, and total acids, or an accurately measured

aliquot of the acids, must be transferred quantitatively to the column. There are approximately 10 to 20 µl of volatile fatty acids in 2 ml of rumen liquor and it is very difficult to obtain an accurate aliquot of this small a sample. For this reason it was decided to search for a method which would provide for the conversion of sodium salts to free volatile fatty acids and for the quantitative transfer of the entire fatty acid mixture to the column. Various techniques have been reported for the introduction of volatile samples into the gas-liquid chromatograph column (44,45,61)

An apparatus which was developed for this purpose is shown in Fig. 3. A portion of helium gas was diverted through this glass apparatus by means of a three-way stopcock (stopcock B). The solution containing sodium salts of volatile acids was lyophilized in tube C. The coiled section of the tube was placed in a dry ice-acetone bath. The four-way stopcock (stopcock D) was set such that the helium passed through this section and thence to the atmosphere by way of the other four-way stopcock (stopcock E). Ninety-six per cent H_3PO_4 was added to tube C from the H_3PO_4 reservoir A. Tube C was placed in an oil bath at 200° C. for 20 minutes. The free volatile fatty acids were trapped in the coiled section of the tubing. This portion of the system was then isolated by proper manipulation of stopcock D. The acids were volatilized by placing this portion of the system in the oil-bath for 15 minutes. The volatile fatty acids were then swept on the column by proper manipulation of the four-way stopcocks. The glass tubing from tube C to stopcock D and from stopcock D to stopcock E was heated with nichrome-wire. The recovery of the volatile acids by this method was consistantly low (of the order of 20%). The relatively low vapor pressure of the short-chain fatty acids in aqueous solution

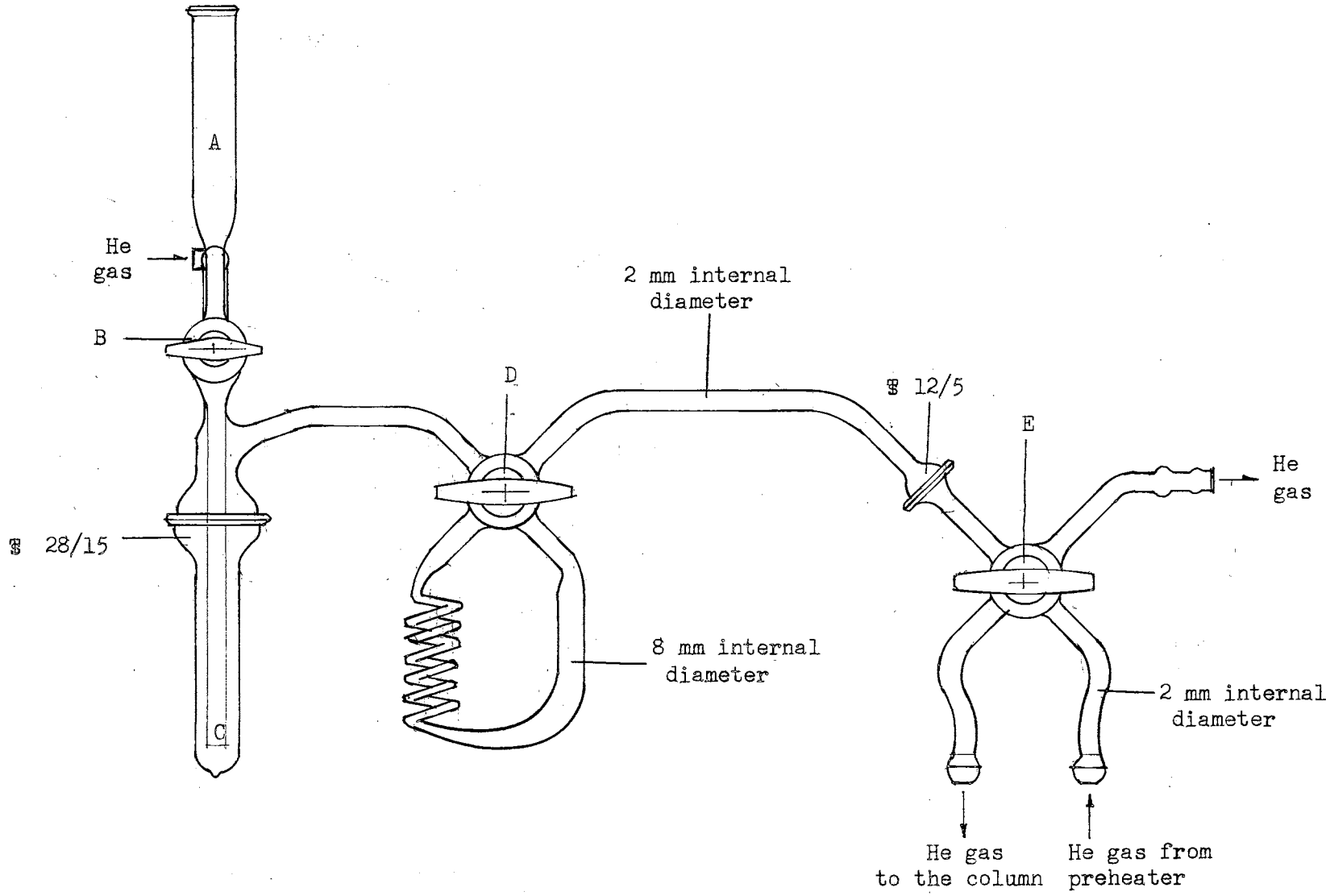

Fig. 3. An Apparatus Used to Introduce Samples on to the Column: A. Reservoir for H_3PO_4; B. Three-way stopcock; C. Reaction vessel; D. and E. Four-way stopcocks.

(63) at atmosphere pressure may offer an explanation for the poor recovery of acids using this system.

Since the volatile fatty acids did not appear to distill in a quantitative manner at atmosphere pressure, a procedure involving vacuum distillation was developed. The system designed for the vacuum distillation is shown in Fig. 4. The system consists of three components. The main component is a specially-designed Y-tube which connects to a high vacuum line through a 19/22 ground-glass standard-taper joint. One limb of the Y-tube terminated with a 28/15 ground-glass ball joint. A small lyophilization tube containing the sodium salts was connected to this limb. The third limb of the Y-tube was connected to a piece of 5 mm pyrex tubing via a 7/15 standard-taper joint. The tube containing the sample was chilled in a liquid nitrogen bath, and 95-97% H_3PO_4 was then added with a pipette to the inner wall of the tube. The tube was once again placed in the liquid nitrogen bath and connected to the Y-tube. The entire system was evacuated to 0.005 mm Hg, and then the system was isolated by closing the stopcock leading to the vacuum line. The liquid nitrogen bath was removed from the sample tube and placed in such a position that the lower tip of the 5 mm tubing was in the cold bath. The sample tube was placed in a warm water bath. The volatile acids were allowed to distill into the collecting tube for 20 minutes. The lower one-inch of the 5 mm collecting tube was sealed into an ampoule by heating the tube with a micro-torch. The volatile fatty acids were thus sealed in an evacuated ampoule.

Transfer of the volatile fatty acids from the ampoule to the column was performed with the aid of the apparatus shown in Fig. 5. The

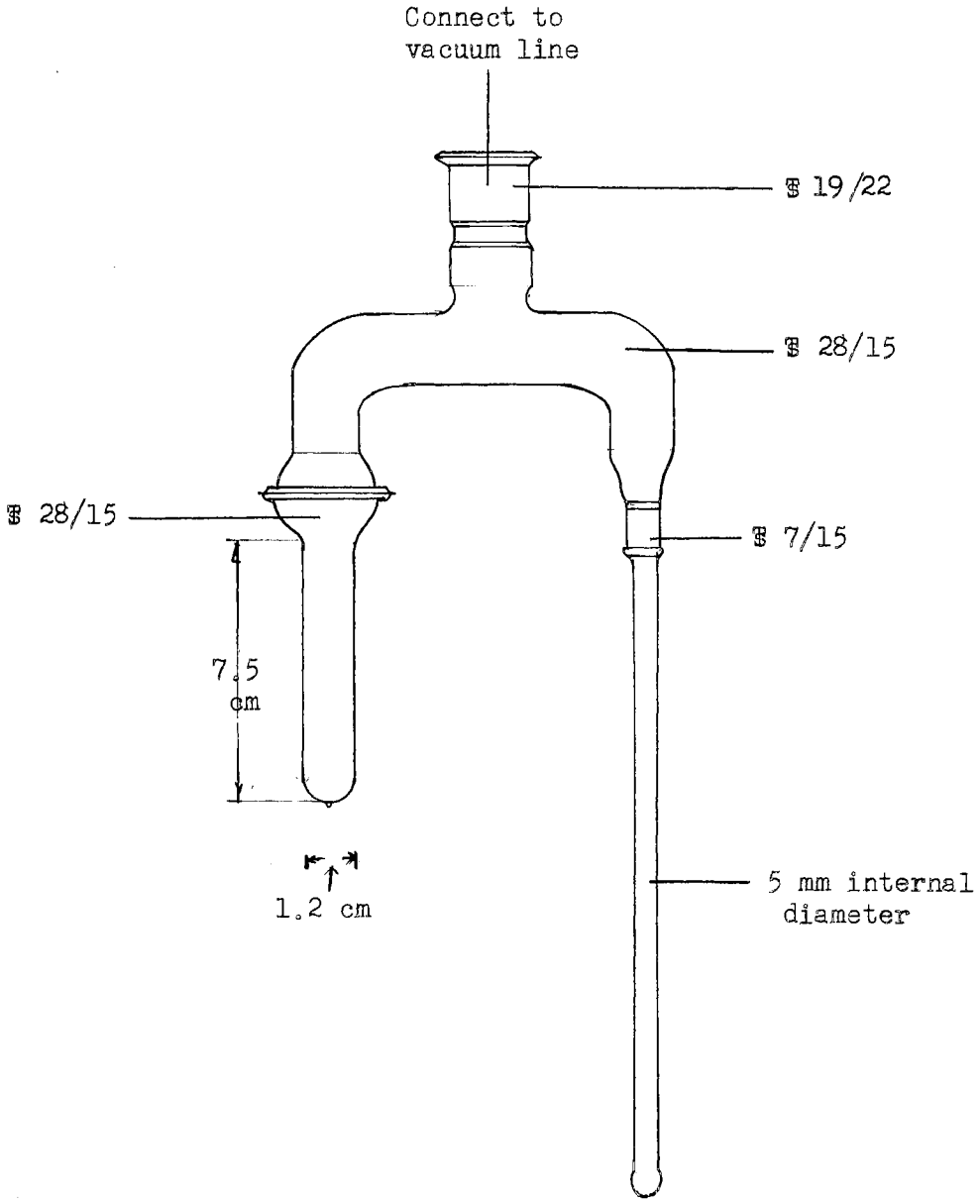
Connect to
vacuum line
⅋ 19/22
⅋ 28/15
⅋ 28/15
⅋ 7/15
7.5
cm
1.2 cm
5 mm internal
diameter

Fig. 4. Y-Tube

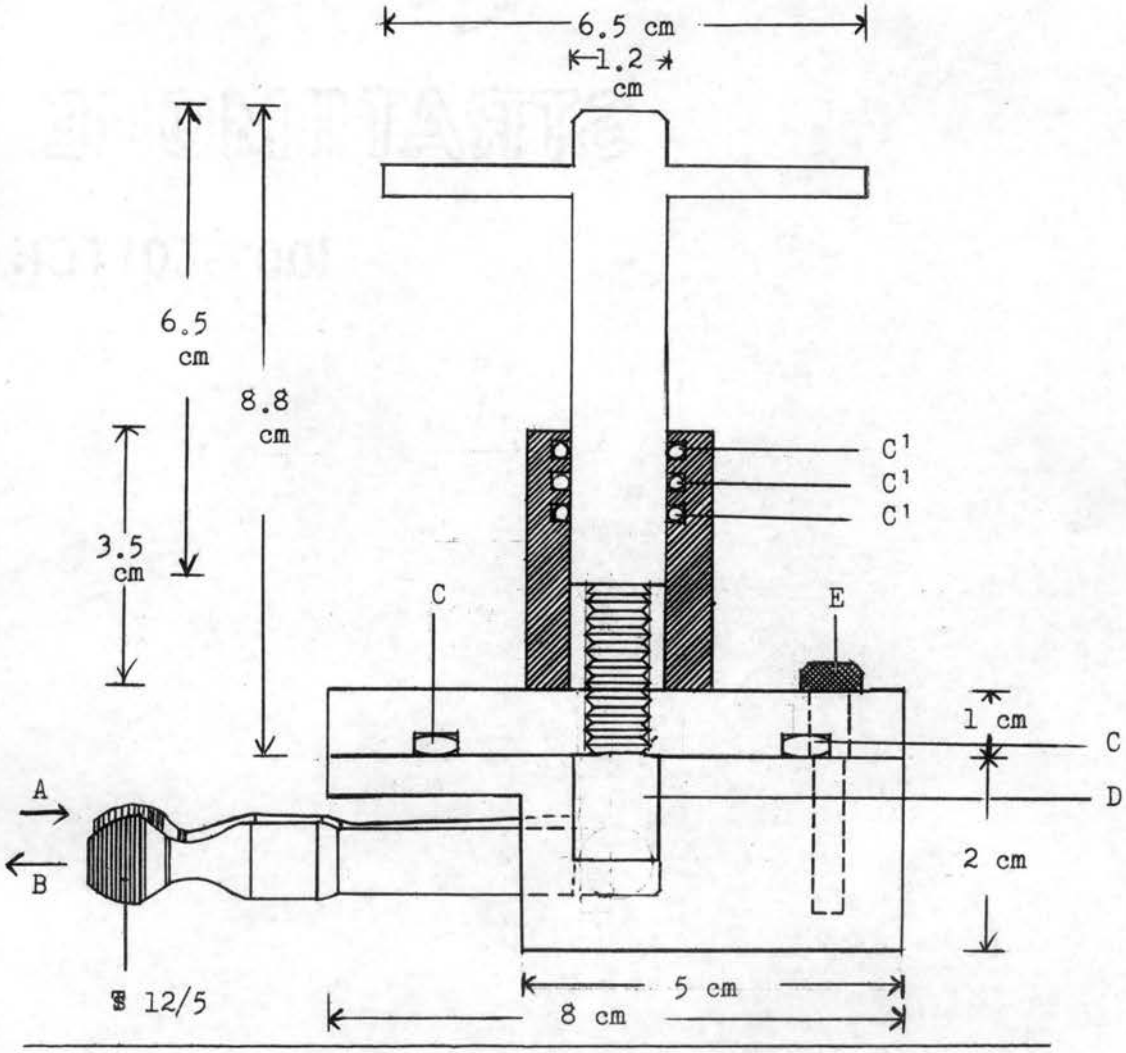

Fig. 5. Cross-Section of a Heating Chamber: A. Helium gas from preheater; B. Helium gas into gas-chromatograph column; C. Silicone rubber ring, parker size 2-327 compound S 418-6; C¹. Silicone rubber ring, parker size 2-112 compound S 418-6 (C and C¹ obtained from Industrial Gasket and Packing Company, Oklahoma City, Oklahoma); D. Ampoule chamber; E. Screw.

system consists of a brass block with a small compartment into which the glass ampoule can be placed. The chamber is connected by tubes to the gas chromatograph preheater and column such that the carrier gas passes through the chamber on its way to the column. The entire brass system was gold-plated to prevent a reaction between the volatile acids and the brass. The entire unit was wound with nichrome heating wire and asbestos. The flow of gas to the column was interrupted while the ampoule was placed in this chamber. The chamber was sealed and the gas-flow once again initiated. The ampoule was heated in the heating chamber for 15 minutes, and then it was broken by applying pressure with the center screw. The heated fatty acid vapors were swept through the column by the carrier gas. The per cent recovery of the volatile fatty acids was determined by measuring the peak areas (the product of peak height times the width at one half peak height (62)) and comparing these values with those obtained from the same quantity of the mixture of the fatty acids injected directly by means of a micro-syringe. Table III shows the per cent recoveries of volatile fatty acids following vacuum distillation of the free acids and their transfer to the column. There appears to be no simple explanation for the poor recovery of formic acid. It is perhaps possible that a mono-molecular layer of formic acid adsorbs to the wall of the Y-tube.

A number of methods were tested for the conversion of the sodium salts to the free fatty acids. Concentrated sulfuric acid was tried, but no free acids were recovered. Concentrated sulfuric acid caused some charring, and it is possible that this strong acid caused destruction of the organic acids. Sodium acetate was added to the reaction flask and treated directly with 100-200 mesh Dowex 50 (hydrogen form) with

TABLE III

PER CENT RECOVERY OF THE MIXTURE OF VOLATILE FATTY ACIDS

AFTER DISTILLATION AND TRANSFER TO COLUMN

Acid	Experiment I	Experiment II	Average
Formic Acid	56.9	55.1	56.0
Acetic Acid	98.3	95.7	97.0
Propionic Acid	95.0	99.4	97.0
Iso-butyric Acid	92.6	91.6	92.1
n-Butyric Acid	86.8	85.0	85.9
Iso-valeric Acid	80.2	73.2	76.5
n-Valeric Acid	77.0	63.4	70.2

The gas chromatography was carried out under the following condition: Column temperature: 156° C; preheater: 230° C.; cell temperature 195° C.; carrier gas: helium; gas flow rate: 60.8 ml/min; attenuation: 5.

the hope that sufficient moisture would be associated with the Dowex particles to insure ionization and ion exchange. This method resulted in the distillation of much water, and the recovery of acetic acid was only 10 per cent. The method which was finally adopted was the use of ortho-phosphoric acid. Eighty-six per cent ortho-phosphoric acid also resulted in the distillation of an excessive quantity of water. Anhydrous phosphoric acid, prepared by addition of phosphorus pentoxide to H_3PO_4 in an amount 10% in excess, was next tried, but the recovery of the volatile fatty acids was found to be low. This low recovery from anhydrous phosphoric acid was possibly due to the high viscosity of the anhydrous phosphoric acid. It may also have been due to association of the organic acids and phosphoric acid or the formation of non-volatile mixed anhydrides. The optimal concentration appeared to be 95-97% H_3PO_4. Table IV shows the recoveries obtained when a mixture of the volatile fatty acids was treated with 95-97% H_3PO_4 during the distillation.

E. Application of Method for Analyses of Rumen Liquor.

An extensive study of the application of this method to analysis of rumen liquor was beyond the scope of the work conducted for this thesis. However, a preliminary test of this application was carried out as follows: Two milliliters of a typical rumen liquor were introduced into the glass lyophilization tube. Table V gives the results obtained when duplicate 2 ml. sample of rumen liquor were carried through the procedure outlined. It is seen that agreement between the duplicate samples is relatively good for each of the fatty acids for which duplicate values were obtained. It is also seen that the actual

TABLE IV

PER CENT RECOVERY OF VOLATILE FATTY ACIDS WITH

95-97% ORTHOPHOSPHORIC ACID

Fatty Acid	Per Cent Recovery Experiment I	Experiment II	Experiment III	Average
Acetate	94.0	84.2	84.0	87.6
Propionate	88.0	90.0	90.0	89.3
Iso-butyrate	86.0	89.0	89.0	88.0
n-Butyrate	69.0	76.0	74.0	73.0
Iso-valerate	54.8	64.7	67.0	62.2
n-Valerate	40.0	43.4	38.0	40.5

The gas chromatography of fatty acids were carried out under the condition as follows: Column temperature: 155° C.; preheater: 230° C.; cell temperature: 200° C.; carrier gas: helium; gas flow rate: 60 ml/min.; attentuation: 5.

TABLE V

ANALYSIS FOR VOLATILE FATTY ACIDS IN RUMEN LIQUOR

	Duplicate A µl/2 ml Sample	Duplicate B µl/2 ml Sample	Average	Apparent Amount µmoles/ ml sample	Corrected Amount*** µmoles/ ml sample
Acetate	7.49**	---*	7.49	65.4	74.5
Propionate	1.37	1.20	1.29	8.65	9.68
Iso-butyrate	0.30	0.30	0.30	1.60	1.82
n-Butyrate	0.97	0.85	0.91	5.90	8.08
Iso-valerate	0.25	0.35	0.30	1.35	2.17
n-Valerate	0.20	0.24	0.22	1.0	2.48

The gas chromatography of fatty acids was carried out under the following conditions: Column temperature: 155° C.; preheater temperature: 230° C.; cell temperature: 200° C.; carrier gas: helium; gas flow rate: 60 ml/min.; attenuation: 5;

* The peak of acetic acid was too high to be measured under attenuation 5

** Recorded at an attenuation of 15.

*** Calculated by application of the per cent recoveries shown in Table IV.

quantities of the different fatty acids are within the range of reasonable expectation. Thus, on the basis of this very preliminary study, it appears that the method is reproducible and that it offers promise for application to small samples of biological materials.

CHAPTER III

SUMMARY AND CONCLUSIONS

1. A method was sought for the separation and quantitative determination of volatile fatty acids which would be applicable for one to two milliliters of rumen liquor or similar biological fluid. Gas-liquid chromatography of the free volatile fatty acids was found to be a satisfactory system for the separation and quantitation.

2. A procedure is described in which the free acids are converted to the sodium salts, and the water is removed by lyophilization. Apparatus was developed for the conversion of sodium salts to the free fatty acids in vacuo and the vacuum distillation of the volatile acids into an ampoule which is subsequently sealed. A second piece of apparatus provides for the transfer of the volatile acids from the ampoule to the gas-liquid chromatograph column.

3. The recovery of the acids by this procedure was variable. In the case of acetic, propionic and also iso-butyric, the recoveries were approximately 90%. The per cent recoveries with n-butyric, iso-valeric and n-valeric were 73, 62 and 40 respectively.

4. The procedure was employed for the analysis of one sample of rumen liquor, and the results obtained appeared to be reproducible.

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