GROWTH AND DEVELOPMENT

With Special Reference to Domestic Animals

XLII.   Methane, Hydrogen, and Carbon Dioxide Production in the Digestive Tract of Ruminants in Relation to the Respiratory Exchange.

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FOREWORD

The special investigation on growth and development is a cooperative enterprise in which the departments of Animal Husbandry, Dairy Husbandry, Agricultural Chemistry, and Poultry Husbandry have each contributed a substantial part. The parts for the investigation in the beginning were inaugurated by a committee including A. C. Ragsdale, E. A. Trowbridge, H. L. Kempster, A. G. Hogan, F. B. Mumford. Samuel Brody served as Chairman of this committee and has been chiefly responsible for the execution of the plans, interpretation of results and the preparation of the publications resulting from this enterprise.

The investigation has been made possible through a grant by the Herman Frasch Foundation, now represented by Dr. F. J. Sievers.

F. B. MUMFORD
Director Agricultural Experiment Station

ABSTRACT

A method is described for securing from cattle directly expired air for analysis. By the employment of this method, time curves after feeding were mapped for expired CO_2 and CH_4 and consumed O_2. These time curves on expired air were paralleled by time curves after feeding on the composition of rumen gas of living animals. Volume and composition of the gas in the various regions of the digestive tract of slaughtered animals were also determined. Assuming equal diffusion rates from the rumen for CO_2 and CH_4, the total CO_2 expired was corrected for the fermentation CO_2 thus enabling the computation of "true" R. Q.s (respiratory quotient) as contrasted to the "apparent" R. Q.s formerly published. These data also made possible corrections for CH_4 accumulated in spirometers (or respiration chambers) during "metabolism tests" and computation of feed energy losses in fermentation gases from the digestive tract, which on maintenance feed intake approximate 25% of the maintenance energy requirement. The absolute fermentation losses are not constant, but decline rapidly with time after feeding. The ratio of rumen CO_2 to CH_4 also declines with time after feeding, due largely to the percentage decline in the CO_2.

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XLII.   METHANE, HYDROGEN, AND CARBON DIOXIDE
PRODUCTION IN THE DIGESTIVE TRACT OF
RUMINANTS IN RELATION TO THE RESPIRATORY
EXCHANGE.*

LLOYD E. WASHBURN AND SAMUEL BRODY

It is generally known that considerable anaerobic carbohydrate fermentation occurs in the rumen, with methane (CH_4) and carbon dioxide (CO_2) as principle end products. The intermediary reactions whereby these end products are formed are not clearly known. Diagrammatically, the reaction may be represented in various ways, such as

$$C_6H_{10}O_5 + H_2O \rightarrow 3\ CO_2 + 3CH_4$$

and

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$
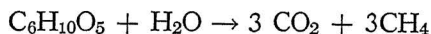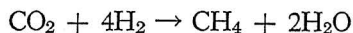
The economic importance of fermentation energy losses is evident from the following considerations. The fuel value of CH_4 is about 9.474 Calories (kilo-calories) per liter. The CH_4 production by a highly fed dairy cow is of the order of 300 liters per day. The fuel loss in the form of CH_4 (and some H_2) is thus about 3000 Calories per day. In addition to the 300 liters CH_4 production, at least as much, that is, 300 liters CO_2 is produced by fermentation. Assuming that the fuel equivalence of CO_2 is of the order of 4.2 Calories per liter, then the loss associated with the fermentation CO_2 is about 1400 Calories per day. Total fermentation energy loss is thus over 4000 Calories per day, equivalent to over half the maintenance needs of the average cow. There are undoubtedly other fermentation energy losses in addition to the energy associated with the fermentation CO_2 and CH_4. These are important economic facts.

Theoretic aspects of the fermentation problem are many sided. The mechanisms and intermediary steps by which the products are formed are not known. Outside of CH_4, CO_2, small quantities of H_2, and fatty and other organic acids, the nature of the fermentation products is not definitely known. The fermentation is essentially anaerobic. The source of oxygen for the process, and the identity of the fermenting

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*This bulletin is based on a dissertation by Lloyd Eugene Washburn presented in May, 1937 to the Graduate School of the University of Missouri in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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organisms are not definitely known. We know that CO_2 is one of the fermentation products. There is also CO_2 formation from the interaction of salivary carbonates or bicarbonates with fatty and other organic acids produced in the fermentation process, about which little is known. From the respiratory exchange point of view, the most important problem is the differentiation between fermentation CO_2 and tissue metabolism CO_2. The computation of the metabolic heat production and the respiratory quotient necessitates knowing metabolism CO_2, distinct from fermentation CO_2. The determination of metabolism from oxygen consumption by the closed-circuit spirometer method necessitates knowing the amount of CH_4 accumulated in the spirometer or chamber during the "metabolism test" thus displacing the O_2 consumed. It is these later considerations that are responsible for the present investigation on fermentation gas production. We felt special need for developing correction factors for the methane in the spirometers, and a general need for clearing up the relation between fermentation gases and respiratory exchange gases, and their bearing on the respiratory quotient, specific dynamic action, and net-energy problems.

II. LITERATURE

While there is relatively little definite information on the various aspects of the fermentation process in ruminants, there is a considerable literature on the problem. Indeed the literature is too large for critical review at this time. Only a few references of particular historical or biochemical interest can be cited.

Methane formation in ruminants was demonstrated in 1875 by Popoff in Hoppe-Seyler's laboratory. The methane formation was attributed to micro-oragnisms, since ruminants digest cellulose in the absence of cytase (Haubner and Sussdorf, 1855; Henneberg and Stohmann, 1864; Wildt, 1874), and since Tieghem (1879) demonstrated cellulose fermentation by *B. amylobacter* (also Scheunert, 1906; Hoesslin and Lesser, 1910, Hopffe, 1919). While, according to Manngold (1929, 1930), protozoa do not digest cellulose.

Tappeiner (1884) reported the production of 4.7 grams CH_4 per 100 grams of cellulose digested. Kellner (1907) reported the formation of 3.17 grams CH_4 per 100 grams of starch digested; 5.45 grams CH_4 per 100 grams straw pulp; 4.29 grams CH_4 per 100 grams mixed ration. Forbes et al (1928) reported (see Mitchell, 1932, also Missouri Res. Bul. 193, 1933) the following methane production in steers per 100 grams of digestible carbohydrate in an alfalfa and corn ration; 5.37 grams on ½-maintenance level; 4.86 on 1-maintenance; 4.32 on 1.5-maintenance; 4.30 on 2-maintenance; 4.37 on 2.5-maintenance; 4.22

on 3.0-maintenance. The total CH_4 production ranged from 58.8 grams per day on $\frac{1}{2}$-maintenance to 230.4 grams per day on 3.0-maintenance.

There is a considerable literature on the influence of various feed combinations and extracts on the fermentation processes. Thus Armsby and Fries (1918) reported that inclusion of starch in the diet resulted in increased methane excretion along with decreased cellulose digestibility (see also Kriss, 1930). The influence of non-protein nitrogen in feed on fermentation was discussed by Zuntz (1891), and investigated by Armsby (1921), and Markoff (1913).
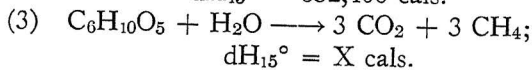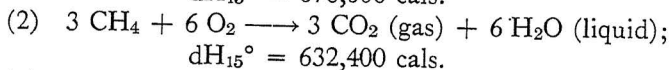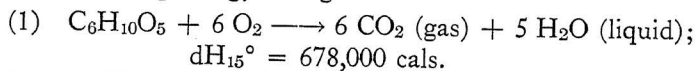
There are also a number of comparative studies on gas production in the digestive tracts of various herbivora and carnivora. (Pettenkofer and Voit, 1871, 1873; Tappeiner, 1884; Colasanti, 1877; von der Heide and Klein, 1913; Zuntz and Lehmann, 1889; Boycott and Damant, 1907). Ritzman, Washburn and Benedict, (1936) reported that the percentage volume of total CH_4 excreted with reference to total CO_2 excretion is 4 for the horse, 7-8 for the cow, 7 for the sheep, 4-6 for the goat; 4 for the elephant (Benedict 1936). In the elephant 25% of the CH_4 is excreted by way of the trunk, and 75% through the anus.

There is a considerable literature on chemical and bacteriological studies of fermentation of cellulose under various conditions. (Hoppe-Seyler, 1886; Omeliansky, 1887, 1902, 1904; Khouvine, 1923; Pringsheim, 1913; Kroulik, 1913; Lymn and Langwell, 1923; Viljoen, Fred, and Peterson, 1926; Kellerman and McBeth, 1913; McBeth and Scales, 1913; Van Senus, 1890; Krabbe, 1890; Winogradsky, 1926; much of this literature has been reviewed by Buchanan and Fulmer (1930).

Perhaps the most extensive studies on the chemistry of methane formation were reported by Buswell and associates. Boruff and Buswell (1929) studied the production of carbon dioxide and methane from anaerobic fermentation of corn stalks, and suggested the equation

$$C_6H_{10}O_5 + H_2O \longrightarrow 3\,CO_2 + 3\,CH_4$$

involving the following energy changes:

(1) $C_6H_{10}O_5 + 6\,O_2 \longrightarrow 6\,CO_2$ (gas) $+ 5\,H_2O$ (liquid);
$$dH_{15}° = 678,000 \text{ cals.}$$

(2) $3\,CH_4 + 6\,O_2 \longrightarrow 3\,CO_2$ (gas) $+ 6\,H_2O$ (liquid);
$$dH_{15}° = 632,400 \text{ cals.}$$

(3) $C_6H_{10}O_5 + H_2O \longrightarrow 3\,CO_2 + 3\,CH_4$;
$$dH_{15}° = X \text{ cals.}$$

Equation (1) $-$ Equation (2) $=$ Equation (3)

or

678,000 cals. $-$ 632,400 cals. $=$ 45,600 cals.

Thus, 45,600 cals. or 6.7 per cent of the total heat of oxidation is consumed in the biological hydrolysis of cellulose to form methane and car-

bon dioxide. Breden and Buswell (1933) found that by the use of shredded asbestos many of the formerly encountered difficulties in culturing the methane forming organisms could be removed. Using this technique, Symons and Buswell (1933) carried out complete anaerobic fermentations of a number of pure substances, with the following CO_2 to CH_4 ratios:

1 : 3	*1 : 2*	*1 : 1*	*3 : 5*
n amyl alcohol	acetone	acetic acid	ethyl acetate
n butyl alcohol		dextrin	acetaldehyde
ethyl alcohol	trimethylene	inulin	
methyl alcohol	glycol	lactic acid	*5 : 7*
propyl alcohol		starch	
isoamyl alcohol		xylose	glycerol
		dextrose	
		lactose	*11 : 13*
		sucrose	
		arabinose	dulcitol
			rhamnose

9 : 7	*7 : 5*	*11 : 5*	*3 : 1*	*7 : 1*
succinic acid	pyruvic acid	tartaric acid	formic acid	oxalic acid

Compounds undergoing fermentation were classified into those adding water, losing water, and neither gaining nor losing water. Calculations showed that if water was the only source of oxygen besides that in the compound which underwent fermentation, carbon dioxide and methane would be the expected end products, since the greatest decrease in free energy occurred with their formation. The fermentation reaction was regarded as one of oxidation and reduction involving the addition of water and decarboxylation, and forming acids, carbon dioxide and hydrogen. It was thought that methane was formed by the combination of hydrogen with some of the carbon dioxide, according to the equation

$$4\,H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O$$
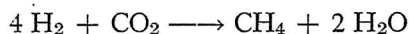
Carbohydrate fermentation was considered to be a stepwise breakdown, possibly forming cellobiose, dextrose, and succinic, butyric, propionic, lactic, pyruvic, acetic, and formic acids as intermediates. Carbon doxide and methane were the two final end products except for a small amount of hydrogen which might have been due to incompleteness of reaction. The following general equations were proposed for the fermentation process:

(1) For carbohydrates,

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \longrightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2$$

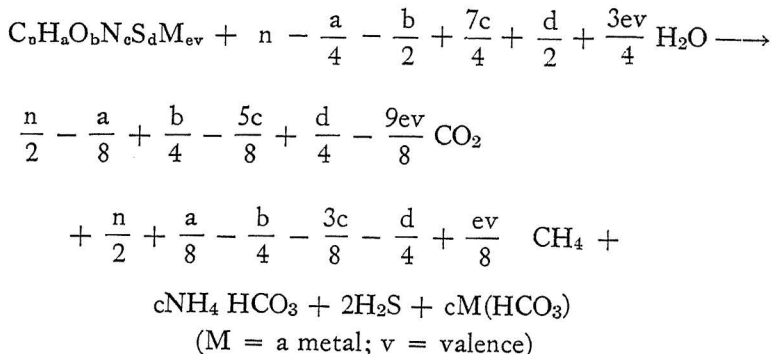$$+ \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4$$
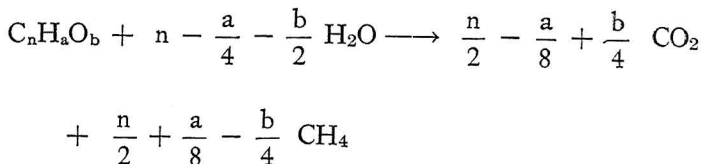
(2) For compounds containing nitrogen, sulphur, and a metal,

$$C_nH_aO_bN_cS_dM_{ev} + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{7c}{4} + \frac{d}{2} + \frac{3ev}{4}\right) H_2O \longrightarrow$$

$$\left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} - \frac{5c}{8} + \frac{d}{4} - \frac{9ev}{8}\right) CO_2$$

$$+ \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} - \frac{d}{4} + \frac{ev}{8}\right) CH_4 +$$

$$cNH_4 HCO_3 + 2H_2S + cM(HCO_3)$$

$$(M = \text{a metal}; v = \text{valence})$$

Methane formation has been investigated quantitatively by Barker (1936). Ethyl alcohol was oxidized to acetic acid, and carbon dioxide equivalent to the methane formed was reduced. With butyl alcohol as a substrate, the results were complicated by further oxidation of the formed butyric acid. It was suggested that methane is always formed through a reduction of carbon dioxide.

Finally there is a literature on analyses of rumen-gas constituents, and on the volume ratio of CO_2 to CH_4. Tappeiner (1883) examined rumen gas from an ox, two goats, and a suckling lamb after slaughtering the animals. He found 45-67% CO_2; 31-34% CH_4; 0.19-0.71% O_2; 0.19-4.7% H_2; 1.9-15.2% N_2. The ratio of CO_2 to CH_4 ranged from 1.3 to 2.2, being highest in the ox, and lowest in the lamb. Lungwitz (1892) analyzed ox-rumen gas secured through a permanent cannula. The CO_2 to CH_4 ratios ranged from 2.1 to 3.5; O_2 content, 0-3%; N_2, 2-19%; CO_2, 40-50% for cabbage leaf feeding and 70-80% for alfalfa, clover and grass; CH_4, 16% after buckwheat, 34% after vetch. Boycott and Damant (1908) reported CO_2 to $CH_4 + H_2$ ratios of 1.9 to 15.2 in the rumens of freshly killed goats and 1.35 to 3.1 in rumens incubated for 41 hours. Markoff (1911) reported CO_2 to CH_4 ratios in goat rumen gas obtained by a needle. The ratios ranged from 0.75 to 3.68. Klein (1916, 1921) reported a CO_2 to CH_4 ratio of 3.68 for the gases excreted by an ox through the esophagus and anus. Mollgaard and Andersen (1917) re-

ported a 2.81 ratio for gas similarly excreted by a dairy cow. These ratios did not include the digestive-tract CO_2 and CH_4 excreted by way of the lungs. (For diffusibility of CO_2 and CH_4 see Haggard and Henderson, 1919; Techendorf, 1922; and McIver, Redfield, and Benedict, 1926). Moreover, these ratios include CO_2 that may have been produced by aerobic fermentation of excreta present in the chamber (See Aubel 1926)

Krogh and Schmidt-Jensen (1920) studied the CO_2 to CH_4 ratio in *in vitro* fermentations of ox rumen contents. Samples of rumen material from slaughtered animals were diluted with buffer solution or water in order to secure small representative aliquots. The fermentations were carried out in 50 cc. fermentation flasks. Gas forming in the flasks was measured by mercury manometers and analyzed with the Schmidt-Jensen micro apparatus (1920). When phosphate buffer was used, the average CO_2 to CH_4 ratio was 2.6 with a range of 2.2 to 2.95. When carbonate was employed as the buffer, the ratios were 4.0 to 4.9. These workers suggested that the 2.6 ratio was representative of the fermentation process in the rumen and that it should be used for correcting respiratory quotients of ruminants. In view of Markoff's statement of the formation of fatty acids having an average molecular weight comparable to butyric acid, they tacitly assumed that the fermentation reaction in the rumen proceeded as follows:

$$2\ C_6H_{10}O_5 \longrightarrow 2\ C_4H_8O_2 + 3\ CO_2 + CH_4$$
Cellulose   Butyric acid
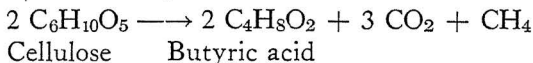
It was pointed out that the CO_2 to CH_4 ratio of 2.6 agreed as closely as could be expected within the limits of experiment with the ratio of 3 shown in the above equation.

While the 2.6 ratio was deduced by Krogh and Schmidt-Jensen from a number of fairly concordant data, it is a question whether it can be applied with certainty in the correction of respiratory quotients. In their experiments, CO_2 and CH_4 were formed in the absence of the buffering effect of carbonates and bicarbonates. In the rumen, however, the fermentation processes are naturally buffered by the saliva, which contains considerable carbonate and bicarbonate as well as phosphate. Moreover, the dilution of rumen contents and the formation of gas against increasing pressure in their experiments hardly represent normal conditions in the rumen of a living animal.

III. AIMS AND METHODS

A. **Aims**—As indicated in the introduction, this investigation was undertaken to solve two pressing practical problems: *1.* Evaluation of CH_4 accumulation in the spirometer (or chamber) to enable correction for the displaced oxygen; *2.* Differentiation between tissue-metabolism

CO_2 and fermentation CO_2 so necessary for interpretation of respiratory exchange data. We were particularly anxious to evaluate the *expired* CH_4, and to map at *short intervals* the time course after feeding of the expired CH_4 and CO_2. Special attention was given to the time course after feeding of the ratio of CO_2 and CH_4 in the digestive tract, because the estimation of fermentation CO_2 (separate from tissue metabolism CO_2) can be made only on the basis of this ratio. Gases in the various parts of the digestive tract of slaughtered animals were also measured and analyzed.

B. **Methods**—These aims necessitated developing a method of securing at short intervals directly expired air in contrast to respiration-chamber air which also contains CH_4 excreted by way of the anus, and that formed by aerobic fermentation of the feces in the chamber. The following paragraphs describe this and the other employed methods in detail.

1. *Gas in the digestive tract of slaughtered animals*: Complete digestive tracts were taken from cows, sheep, and goats. Dairy and beef cattle of unknown history were killed at a packing plant. Rambouliet ewes were slaughtered by the Animal Husbandry Department. Angora goats were slaughtered at the laboratory at different times after feeding a maintenance ration of alfalfa hay and grain mix.

The esophageal and anal openings of the digestive tract were closed by tying the esophagus and rectum securely with heavy cord. In the cow and sheep experiments, the tyings were made after removal of the tract from the body; in the goats, before removal of the tract from the body. By tying in a similar manner between omasum and abomasum, at the pyloric sphincter, and at the ileo-cecal junction, the following segments of the tract were separately closed off: (a) rumen, reticulum and omasum together; (b) abomasum; (c) small intestine, from the pyloric sphincter to the ileo-cecal junction; (d) large intestine, from the ileo-cecal junction to the anus.

After the digestive tract was tied off, gas within each segment was worked to one spot by squeezing and kneading. The gas was removed quantitatively by evacuation with a mercury gas pump or with a glass syringe (B-D Yale; "Luer-Lok" Needle). Large gas volumes, such as found in the rumens, were measured by pumping through the mercury gas pump into a gasometer. After the mercury pump had been washed out several times with rumen gas, a sample of the gas was drawn into the pump and stored for analysis. Evacuation of the rumen gas was completed with a metal pump having an adjustable and non-leakable piston. Small gas volumes were measured and sampled with a syringe prepared as follows:

The syringe and puncturing needle were connected through a short piece of rubber tubing bearing a screw clamp. Before punc-

turing the organ wall for gas evacuation, about 5 cc. of a 1 per cent sulphuric acid solution was drawn through the needle and tubing into the syringe. The acid solution was then slowly expelled, care being taken that all air bubbles were forced out during the process. In this way the needle and tubing were filled with solution, and air was excluded from the system. This technique made it unnecessary to wash the syringe several times with intestinal gas prior to sampling. The gas sample was drawn into the syringe, and while the needle still penetrated the organ wall the volume was read off before closing the screw clamp. A correction was made for the small amount of acid solution drawn during sampling from the needle and tubing into the syringe.

All gas volume measurements were reduced to conditions of standard temperature and pressure.

2. *Rumen gas in living animals*: Gas samples were drawn through permanent rumen cannulae from the rumens of a non-lactating Jersey cow and a lactating Angora goat. The rumen cannula was a long B-D Yale hypodermic needle which was introduced through the left abdominal and rumen walls into the upper part of the rumen. The needle was kept in place by stitching the rumen wall to the abdominal wall, and by tying the stitching thread to the enlarged end of the needle outside the body. The cannula was kept closed with a rubber stopper except when gas samples were taken. Gas was sampled with a vaselined glass syringe connected to the cannula by a metal adapter. A two-way stopcock was interposed between the adapter and syringe to permit washing it with rumen gas. The syringe was washed 3 times, by filling with rumen gas and then expelling the gas outside the body of the animal.

3. *Methane in expired air*: Pulmonary and esophageal excretion of methane and respiratory gas exchange were measured with an open-circuit respiration apparatus. Dairy cows, trained to lie down and remain in a state of muscular repose at the command of the operator, were connected with the apparatus by a mask (described in Missouri Research Bulletin 143) as shown in Figure 1. The animals inhaled pure outdoor air during 30 minute respiratory periods, usually carried out at two-hour intervals after feeding. Directly expired air was measured and analyzed for carbon dioxide, oxygen, and methane. From these measurements the respiratory quotient, methane excretion, and energy production were computed.

4. *The Open-Circuit metabolism apparatus*: After the animal had lain down before the apparatus, outdoor air was forced in through pipe A shown in Figs. 1 and 2, by starting the outdoor air blower. The mask was then placed over the muzzle. Negative pressure set up in the mask and connecting tubes by inspiratory effort caused outlet valves E to close and inlet valves C to open. Outdoor air was drawn into the mask. During expiration, positive pressure in the mask and tubes closed the in-
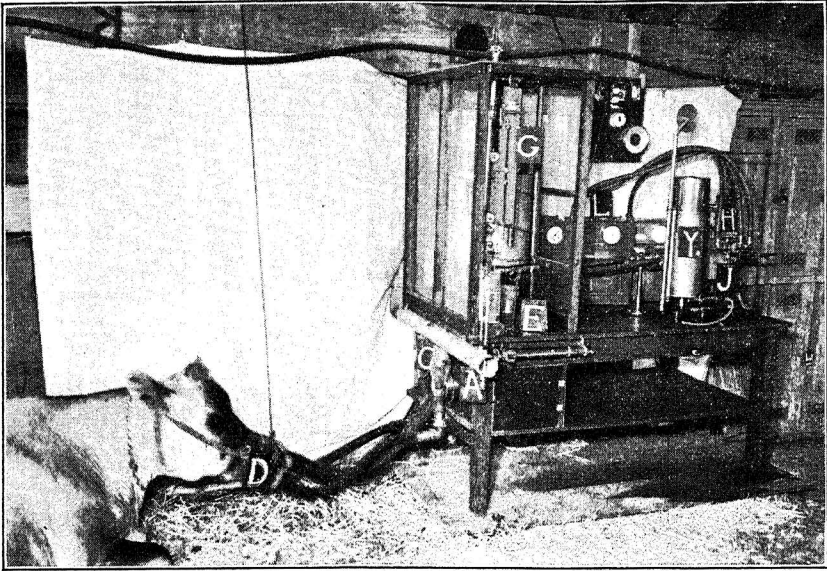
Fig. 1. Cow connected to open-circuit respiration apparatus. For further details see legend for Figure 2 and text.

let valves and opened the outlet valves, thus forcing expired air from the mask into the spirometer F. Filling of the spirometer automatically closed the circuit for the ventilating motor at H by dropping the upper electrode of resistance R_1 into the $CuSO_4$ solution (See automatic ventilation regulator Fig. 2b). Air was then drawn from the spirometer and forced through the meters by the ventilating fan. Excursions of the spirometer bell caused the counter-balance bar T to move back and forth between the bars P of the automatic ventilation regulator. Excessive ventilation caused the spirometer bell to fall, automatically increasing the resistance at R_1 through the upward movement of the spirometer counter-balance and ventilation regulator. If air was expired into the spirometer faster than it was removed by the ventilating fan, a downward movement of the ventilation regulator decreased the resistance at R_1. Because of the construction of the outlet valves E (Fig. 2c) air could not be drawn from the mask by the ventilating fan. By proper adjustment of the bars P for the tidal air of the animal, a ventilation current of considerable constancy was obtained. The resistance R_2 was used for adjustment only in case of extreme changes in the respiratory activity of the animal, or when the bars P were not properly set. During the first five minutes of operation, the aliquoting spirometer Y was washed with expired air by filling and emptying several times. The respiration period was started by taking the meter readings, opening and adjusting

FIG. 2-a
SCHEMATIC DIAGRAM OF OPEN CIRCUIT
RESPIRATION APPARATUS

FIG. 2-b
DETAILS OF AUTOMATIC REGULATOR
FOR VENTILATING FAN

FIG. 2-c
DETAILS OF OUTLET VALVE
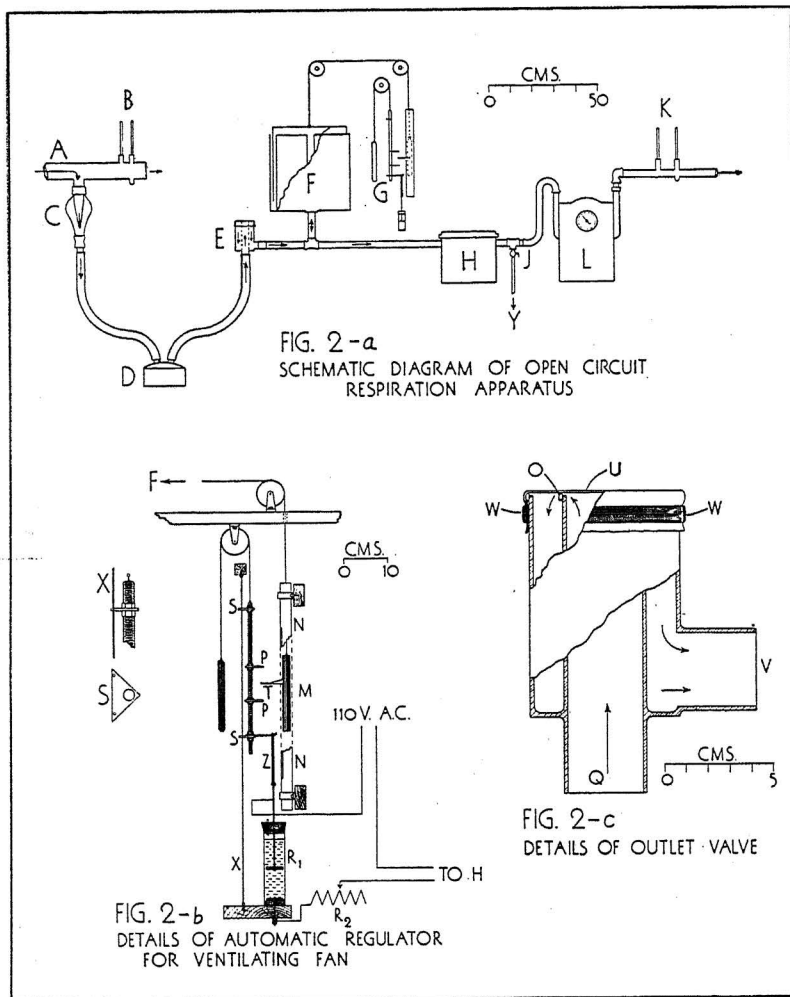
Fig. 2. Diagram with details of the photograph in Figure 1. A, Outdoor air pipe; B, Wet and dry bulb thermometers for outdoor air; C, Intake valve assembly, showing rubber flutter valve inside glass housing; D, Mask; E, Outlet valve; F, Spirometer; G, Automatic resistance controlling the ventilating motor; H, Sealed metal box containing ventilating fan and motor; J, Pet-cock regulating current of air to aliquoting spirometer; L, Meter; K, Wet and dry bulb thermometers for expired air; Y, Aliquoting spirometer; M, Counterbalance for bell of spirometer F; N, Guide for counterbalance M; P, Adjustable bars regulating the movement of the automatic resistance when contacted by T; R_1, Resistance-brass electrodes, one of which moves freely in a dilute solution of copper sulphate—operated by the movement of automatic ventilation regulator; R_2, Resistance (sliding contact tube rheostat) in series with R_1 and ventilating motor—used to help control ventilation only during extreme changes in respiratory activity of the animal; S, Guides for the automatic ventilation regulator; T, Bar attached to counterbalance M—causes movement of automatic ventilation regulator; X, Guide wire for S; O, Band of rubber around end of pipe Q making air-tight seal between dental dam U and pipe Q during inspiration; Q, Inlet pipe; U, Thin dental dam; V, Outlet pipe to spirometer F; W, Broad tight-fitting rubber band holding U in place.

aliquoting spirometer pet-cock J, and taking the readings of the wet and dry bulb thermometers for the outdoor and expired air currents. The period was ended by reading the thermometers, closing the aliquoting spirometer pet-cock, and removing the mask from the animal. Ventilation was stopped automatically through breaking of the circuit in resistance R_1. The final meter readings were then taken.

5. *Gas analysis:* All gas samples were analyzed by the same person with the Haldane apparatus modified by the use of 30 ml. Shepherd combustion pipettes (1931). Measuring burettes were calibrated with mercury. The apparatus was checked periodically by analysis of outdoor air. Carbon dioxide was absorbed in 20 per cent potassium hydroxide solution. Oxygen was absorbed in a solution made by dissolving 10 gms. of pyrogallic acid in 100 cc. of potassium hydroxide solution (KOH and distilled water, 1 : 1 by weight). Methane and hydrogen were burned by passing the gas mixture over a glowing platinum wire in the combustion pipette. Rumen and intestinal gas samples were diluted in the apparatus with outdoor air (about 1/20) in order to (a) supply sufficient oxygen for the combustion of methane and hydrogen; (b) reduce high concentration of carbon dioxide and combustible gases so that they could be measured; (c) economize on the absorbing solutions. Absorptions and combustions were repeated until check readings were obtained. The percentage of each constituent in the gas mixture was determined by dividing the volume of the constituent by the total volume of the sample and multiplying by 100.

6. *Computations:*

(1) Per cent $CO_2 = \dfrac{\text{Volume of } CO_2 \text{ in sample}}{\text{Total volume of gas sample}} \times 100.$

Correction was made for 0.03 per cent CO_2 in outdoor air.

(2) Per cent $O_2 = \dfrac{\substack{\text{Vol. of } O_2 + \text{Vol. of } O_2 \text{ used} \\ \text{absorbed} \quad \text{in combustion}}}{\text{Total volume of gas sample}} \times 100$

(3) Per cent $CH_4 = \dfrac{\text{Vol. of } CO_2 \text{ formed in combustion}}{\text{Total volume of gas sample}} \times 100.$
(Hydrogen absent)

or

$\dfrac{\text{Vol. shrinkage due to combustion}}{2 \text{ (Total volume of gas sample)}} \times 100,$

or

$$\frac{\dfrac{\text{Vol. shrinkage}}{\text{due to combustion}} + \dfrac{\text{Vol. } CO_2 \text{ formed}}{\text{in combustion}}}{3 \text{ (Total volume of gas sample)}} \times 100,$$

since two volumes disappear in the combustion:
$$1\, CH_4 + 2\, O_2 \longrightarrow 1\, CO_2 + 2\, H_2O$$
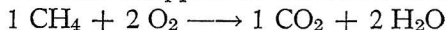
$$\begin{array}{l}\text{Per cent } CH_4 \\ \text{(Hydrogen Present)}\end{array} = \frac{\text{Vol. of } CO_2 \text{ formed in combustion}}{\text{Total volume of gas sample}} \times 100,$$

or

$$\frac{2\left(\dfrac{\text{Volume shrinkage}}{\text{due to combustion}}\right) - \dfrac{CO_2 \text{ formed}}{\text{in combustion}} - 3 \text{ (Vol. of } H_2)}{3 \text{ (Total volume of gas sample)}} \times 100$$
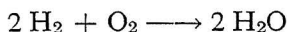
since
$$2\, H_2 + O_2 \longrightarrow 2\, H_2O$$
and 3 volumes disappear in the combustion
and, $1 CH_4 + 2\, O_2 \longrightarrow 1\, CO_2 + 2\, H_2O$
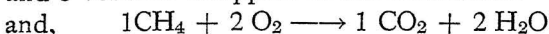
(4) Per cent $H_2 = \dfrac{2}{3} \times \dfrac{\dfrac{\text{Vol. shrinkage}}{\text{due to combustion}} - \dfrac{2\, CO_2 \text{ formed in}}{\text{combustion}}}{\text{Total volume of gas sample}} \times 100$

(5) Percent N_2 was found by subtracting the sum of the volumes of all other gases in the sample from the total volume of the sample, dividing by the total volume of the sample, and multiplying by 100.
     (*Note:* Corrections were made for outdoor oxygen and nitrogen introduced in the dilution of rumen or intestinal gases with outdoor air.)

(6)   R. Q. $= \dfrac{\text{Per cent } CO_2 \text{ expired} - \text{per cent } CO_2 \text{ inspired}}{\text{Per cent } O_2 \text{ inspired} - \text{per cent } O_2 \text{ expired}}$

Inspired oxygen was computed from the equation
$$\frac{\text{Per cent } N_2}{\text{Outdoor air}} : \frac{\text{Per cent } N_2}{\text{Expired air}} = \frac{\text{Per cent } O_2}{\text{Outdoor air}} : \frac{\text{Per cent } O_2}{\text{Inspired air}}$$

(7) CO_2 : Combustible gas ratio $= \dfrac{\text{Per cent } CO_2 - \tfrac{1}{4} \text{ (Per cent } H_2)}{\text{Per cent } CH_4 + \tfrac{1}{4} \text{ (Per cent } H_2)}$

This was based on the assumption that CH_4 was formed by the combination of CO_2 and H_2
$$CO_2 + 4\, H_2 \longrightarrow CH_4 + 2\, H_2O$$
and that hydrogen was present because of incompleteness of reaction.
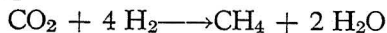
(8) Heat prod. = Total expired air x Per cent O_2 x Caloric value
            corrected to STP   deficit   of O_2 at pre-
                                              vailing R. Q.

RESULTS

In addition to the results on the gas content and composition of gases in the digestive tract, partial data on which are also found in the literature, this bulletin presents the following three essentially new contributions.

1. One rather new contribution is the development of a mask-spirometer method for securing directly expired air from cattle for analysis of CO_2, O_2, and CH_4. Previously published analyses for CH_4 excreted by cattle were carried out on respiration-chamber air in which the expired air was diluted with outdoor air to the extent of about 70%. The chamber air moreover contained gases excreted by way of the anus, and gases formed from the excreta in the chamber. The analyses here reported, on the other hand, were made on expired air uncontaminated by chamber or outdoor air.

2. This new mask-spirometer method made possible the analysis of expired air collected during very short intervals. The method for analysis at short intervals permitted accurate mapping of the *time course* after feeding, not only of O_2 consumption and CO_2 expiration, but also CH_4 expiration. Previously reported analyses of CH_4 for cows were for 12 hour intervals on respiration chamber air only. The new mask-spirometer method permits easy CH_4 determinations for 30-minute intervals. The second new contribution, then, is time curves of CO_2 and CH_4 exhalation and O_2 consumption.

3. With the *same animal at the same time*, these time curves on expired air were paralleled by time curves on the composition of rumen gas and on the CO_2 to CH_4 ratios (more accurately, CO_2 to combustible gas ratios, since in addition to CH_4 there is some H_2 and possibly other less known combustible gases). Such curves on expired and rumen gas in parallel are also new.

A. **Living animals:** The investigation of expired air and rumen gas in parallel was carried out on our regular, well-trained "metabolism cow" Jersey No. 831. She was dry, not pregnant, and weighed about 906 pounds during this period. Three sets of data were obtained on cow 831: one set when she was on a maintenance diet of alfalfa hay; a second, on alfalfa hay and grain; a third on an approximately 2-maintenance grass diet*

The results of these ratios for cow 831 are presented in Tables 1 and 3, and in Figs. 3 to 5. Deductive corrections of the respiratory quotients for fermentation CO_2 are presented in Table 5. Rumen gas data on cow 831 in Table 1 are supplemented with similar rumen-gas data on goat 605 in Table 2.

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*Grass fed *ad lib.* Animal consumed roughly twice the dry matter she had received previously on maintenance hay diet. This was done so that animal would be quiet during respiration experiments.

TABLE 1.—COMPOSITION OF RUMEN GAS OF COW 831, WEIGHT 906 POUNDS, AT DIFFERENT TIMES AFTER FEEDING

Time from Feeding Hrs. Mins.	Percentages of CO$_2$	CH$_4$	O$_2$	H$_2$	N$_2$	Ratios CO$_2$:Combustible Gas	$\frac{N_2}{O_2}$	$\frac{CH_4}{H_2}$	$\frac{CO_2}{O_2}$
3/24/37 to 3/25/37, Fed Alfalfa Hay									
Just before	33.47	35.87	2.41	0.00	28.26	0.93	11.7	----	13.9
30 min. after beginning	36.44	31.64	2.83	1.13	27.97	1.15	9.9	28.0	12.9
7	58.25	22.25	4.50	0.33	14.67	2.62	3.3	67.4	12.9
1 55	67.46	29.94	0.00	2.60	00.00	2.25	----	11.5	----
4	62.59	28.72	3.91	0.00	4.79	2.18	1.2	----	16.0
5 55	63.40	31.06	0.57	0.00	4.97	2.04	0.9	----	10.9
7 35	67.81	32.19	0.00	0.00	0.00	2.11	----	----	----
10 25	49.53	34.11	2.34	0.00	14.02	1.45	6.0	----	21.2
12 45	41.29	40.67	0.00	1.56	16.49	1.02	----	26.1	----
15 55	28.48	37.03	3.34	1.81	29.35	0.77	8.8	20.5	8.5
4/3/37 to 4/4/37, Fed Alfalfa Hay and Grain Mix									
Before feeding	48.50	36.33	0.00	5.64	9.52	1.34	----	6.4	----
Still eating	54.28	33.41	2.26	2.44	7.52	1.62	3.3	13.7	23.9
50	63.34	32.68	0.00	3.98	0.00	1.94	----	8.2	----
2 54	66.24	29.05	0.00	4.71	0.00	2.28	----	6.2	----
4 30	65.88	29.30	0.00	4.82	0.00	2.25	----	6.1	----
6 55	64.69	31.01	0.00	4.30	0.00	2.09	----	7.2	----
8 50	59.26	34.10	0.53	3.06	3.06	1.74	5.8	11.1	111.8
13 42	41.06	32.76	4.50	4.15	17.53	1.25	3.9	7.9	91.2
15 50	34.20	35.10	2.96	2.46	25.29	0.97	8.5	14.2	11.6
17 45	26.47	40.58	5.16	2.44	25.36	0.65	4.9	16.6	51.2
20 35	20.51	38.06	7.30	0.00	34.13	0.54	4.7	----	28.1
23 30	19.88	41.52	3.43	2.34	32.83	0.48	9.6	17.7	58.0
4/8/37 to 4/9/37, Fed Grass									
Before feeding	44.28	39.31	2.37	2.05	11.99	1.13	5.1	19.2	18.6
35 min. after	43.47	23.64	4.55	5.07	23.28	1.84	5.1	4.7	9.6
1 1	80.33	19.67	0.00	0.00	0.00	4.08	----	----	----
2 5	77.56	22.44	0.00	0.00	0.00	3.46	----	----	----
4 15	69.06	29.51	1.43	0.00	0.00	2.34	----	----	48.2
6 5	66.93	32.15	0.00	0.92	0.00	2.08	----	35.0	----
8 10	66.42	32.46	0.75	0.00	0.38	2.05	0.5	----	88.6
11 10	61.81	29.91	1.38	0.00	6.90	2.07	5.0	----	44.8
14 15	40.55	30.32	8.69	0.00	20.44	1.34	2.4	----	4.7
18 10	13.82	15.67	13.27	4.65	52.49	0.88	4.0	3.4	1.0
23 10	10.18	8.95	13.09	0.00	67.79	1.14	5.2	----	0.8

TABLE 2.—COMPOSITION OF RUMEN GAS IN GOAT 605, WEIGHT ABOUT 90 POUNDS, AT DIFFERENT TIMES AFTER FEEDING ALFALFA HAY AND GRAIN.

Time from Feeding		Percentages of					Ratios			
Hrs.	Mins.	CO_2	CH_4	O_2	H_2	N_2	CO_2 : Combustible Gas	$\dfrac{N_2}{O_2}$	$\dfrac{CH_4}{H_2}$	$\dfrac{CO_2}{O_2}$
					Experiment I—2/27/37 to 2/28/37					
	50	49.03	40.06	3.06	2.82	5.03	1.18	1.8	13.1	17.4
2	50	54.81	40.90	1.64	2.79	0.00	1.32	----	24.9	19.7
4	5	54.92	41.34	0.00	3.74	0.00	1.33	----	----	14.7
8	5	41.14	50.00	0.00	9.11	0.00	0.82	----	----	4.5
12	0	31.52	38.10	4.08	9.06	17.25	0.78	1.9	9.3	3.5
24	0	16.70	44.33	1.93	3.43	33.62	0.36	9.8	23.0	4.9
					Experiment II—2/28/37 to 3/1/37					
1	30	33.66	30.53	0.00	6.46	29.35	1.10	4.5	----	5.2
4	10	44.29	47.63	1.95	2.79	3.34	0.91	1.2	24.3	15.9
6	0	49.78	40.39	2.40	0.44	6.99	1.20	15.9	16.8	113.1
8	0	55.43	38.29	0.00	0.00	6.28	1.45	----	----	----
12	0	30.68	39.77	0.00	2.96	26.59	0.77	9.0	----	10.4
24	0	17.53	35.23	1.84	7.51	37.90	0.48	5.1	19.2	2.3

TABLE 3.—RESPIRATION DATA ON COW 831, WEIGHT 906 POUNDS.

Time After Feeding		CO_2 Increment %	O_2 Deficit %	CH_4 %	Apparent R. Q.	CH_4 Lit./hr.	Heat Prod. Cal./hr.	Total Expired Air Lit./hr
Hrs.	Mins.							
3/24/37 to 3,25/37 Fed Alfalfa Hay								
	35	2.67	1.86	0.29	1.44	8.5	276.8	2955
3	15	2.62	2.35	0.26	1.11	6.7	307.4	2587
4	55	2.65	2.38	0.19	1.12	4.8	296.5	2763
9	35	2.58	2.74	0.08	0.94	1.7	292.0	2142
12	0	2.46	1.91	0.03	0.84	0.7	204.8	2121
15	0	2.49	2.90	0.03	0.86	0.6	261.4	1852
21	0	2.47	2.65		0.93	0.9	278.7	1979
4/3/37 to 4/4/37, Fed Alfalfa Hay and Grain Mixture								
2	15	2.87	2.27	0.35	1.27	10.3	337.1	2949
3	20	2.39	1.84	0.29	1.30	9.1	293.6	3166
5	55	2.57	2.25	0.21	1.14	5.5	293.1	3579
8	55	2.39	1.87	0.23	1.28	6.4	266.8	2834
11	10	2.26	2.10	0.13	1.08	4.0	336.0	3174
14	12	2.42	2.62	0.13	0.92	2.9	297.9	2296
17	8	2.53	2.71	0.08	0.93	1.9	316.6	2353
19	0	2.27	2.43	0.09	0.94	1.9	244.3	2027
19	52	2.25	2.59	0.07	0.87	1.1	204.1	1614
22	51	2.47	2.92	0.04	0.85	0.8	265.6	1938
4/8/37 to 4/9/37, Fed Grass								
1	25	2.68	1.71	0.24	1.57	9.8	355.9	4134
3	30	2.54	1.68	0.21	1.51	8.3	340.1	4006
5	35	2.52	2.19	0.22	1.45	6.5	332.9	3007
7	30	2.47	2.24	0.19	1.10	5.9	359.5	3182
10	34	2.66	2.66	0.24	1.00	6.9	388.7	2899
13	35	2.40	2.65	0.10	0.90	2.2	276.4	1764
17	30	2.58	3.03	0.08	0.85	1.8	312.3	2118
22	30	2.62	3.42	0.04	0.77	0.8	306.8	1886
22	32	2.67	3.25	0.07	0.82	1.3	277.7	1807

Figs. 3 to 5 indicate that the levels of the time curves differ with the nature of the diet. They show that the ratio of CO_2 to CH_4 in the rumen is not a constant, as is generally *assumed* (factual data on this ratio for cows, at short intervals over a period of 15 to 24 hours have not been previously published), but that it declines with time after feeding. On the hay ration (Fig. 3, Table 1) ,the ratio CO_2 to CH_4 declined from 2.6 immediately after feeding to O.77, 15 hours after feeding. On the alfalfa hay-grain ration (Fig. 4, Table 1), the CO_2/CH_4 ratio declined from 2.6 immediately after feeding to 0.97, 15 hours thereafter, and to 0.48, 23 hours after feeding. On grass (Fig. 5, Table 1), the CO_2/CH_4 ratio after feeding was at an unexpectedly high level, namely 4.1, declining then to a minimum of 0.9 about 18 hours after feeding. Declines in the ratio of a less spectacular nature were also obtained on goat 605 (Table 2).

The shape of the time curve of the CO_2 to CH_4 ratio in the rumen reflects not so much changes in the time curve of rumen CH_4, as in that of rumen CO_2. Figs. 3 to 5 show that the rumen CH_4 level is relatively constant, of the order of 30%. On the hay, or hay and grain diet (Figs. 3-4, Table 1), the CO_2 level is between 60 and 70% during about 7 hours after feeding; this 7-hour constant level is followed by a rapid decline to 30%, 15 hours after feeding, and to 20% about 20 hours after feeding. On the grass diet (Fig. 5), the initial CO_2 level is at an
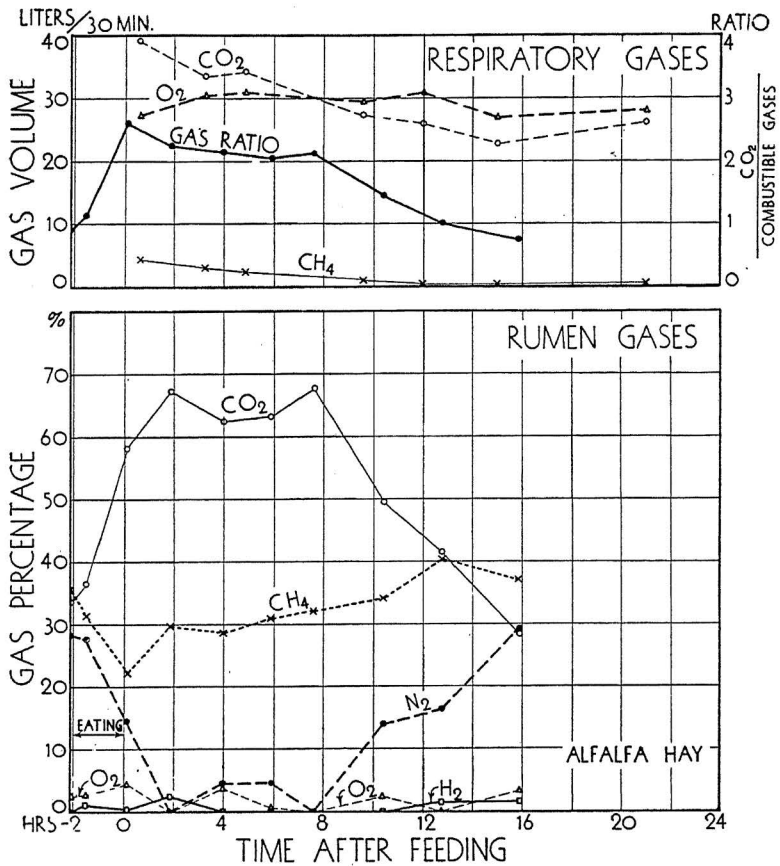
Fig. 3. Time course of the composition of rumen gas (lower quadrant); of the CO_2, O_2 and CH_4 expired and of the CO_2 to CH_4 ratio in the rumen (upper quadrant). The data were secured on a Jersey cow No. 831, fed maintenance ration of alfalfa hay.

80% peak, which declines rapidly to 40% 15 hours after feeding, and 10%, 23 hours after feeding. The meaning of the unusually high initial level of rumen CO_2 following grass feeding is not clear. The consumption of grass of high moisture content is supposed to stimulate less of salivary secretion than does dry feed. Salivary bicarbonate and carbonate could not therefore be the cause of the relatively high CO_2 level following grass feeding. It is also cogent to note that rumination, with its salivary secretion, did not increase the rumen CO_2 level. The salivary origin of rumen CO_2 may perhaps not be as important as one might expect.

Small quantities of H_2 were nearly always found in the rumen gas. The N_2 and O_2 time trends were opposite to that of CO_2. Rumen O_2
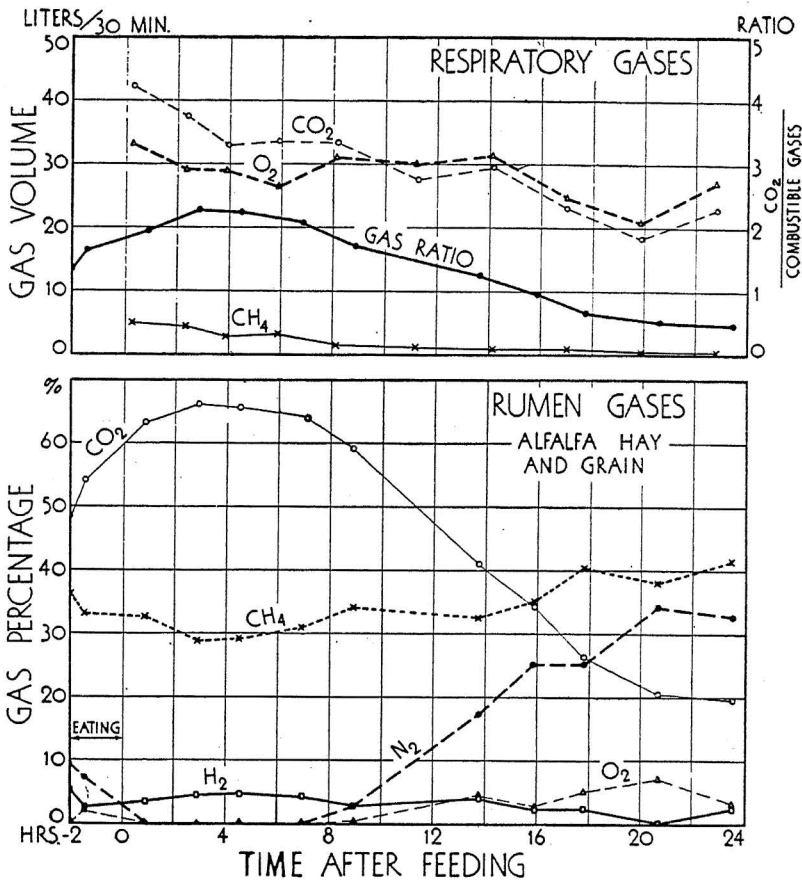
Fig. 4. Same as in Fig. 3, but on a ration of alfalfa hay and grain.

increased slightly during eating, perhaps due to swallowing of air, then decreased immediately, often to zero. The O_2 may be used up in aerobic fermentation. Nitrogen always increased at a more rapid rate than oxygen. It is unlikely that the increase in oxygen and nitrogen percentages is due only to diffusion of these gases into the rumen. According to Teschendorf (1922) oxygen diffuses about three times as fast as nitrogen. Hence, if diffusion were the only process taking place along with increasing percentages of oxygen and nitrogen, the percentage of oxygen should be about three times that of the nitrogen. These data show, on the contrary, that nitrogen exceeds the oxygen by a ratio greater than 3 : 1, from 12 hours after feeding to the end of the experimental period. Of course it is possible that oxygen may be used
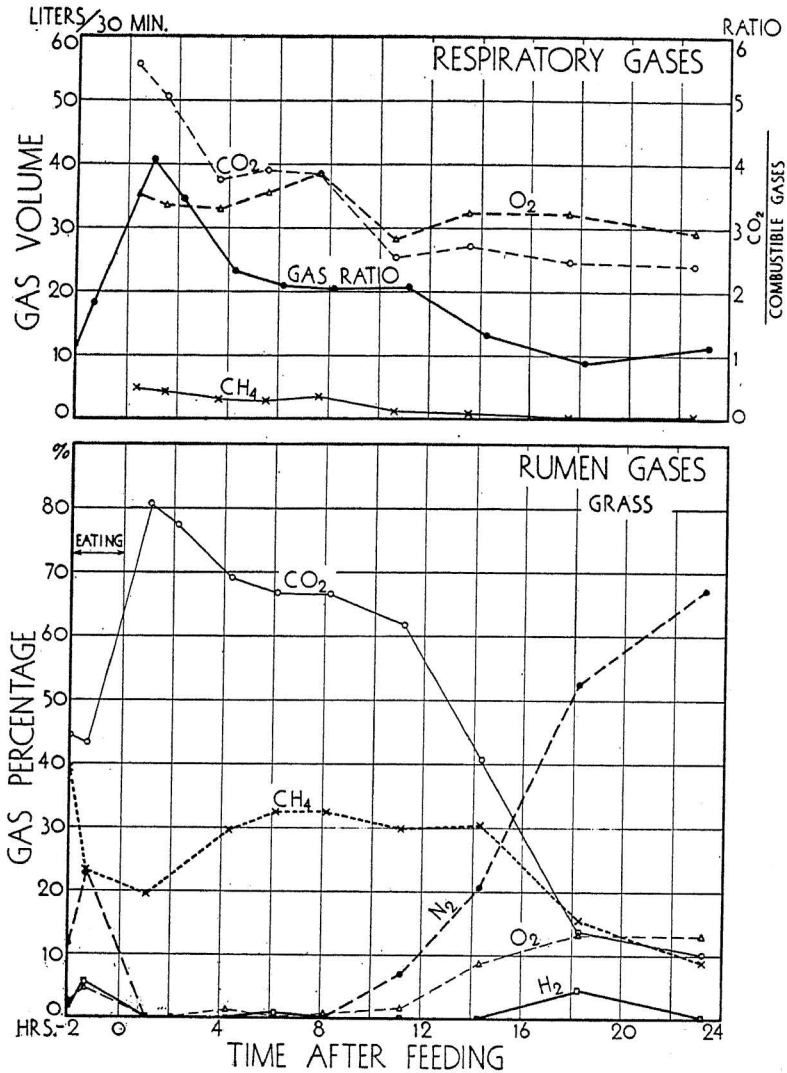
Fig. 5. Same as in Fig. 3, but on a grass diet.

in aerobic processes. This, however, is hardly a plausible explanation in the face of a rapidly falling carbon dioxide percentage.

Table 3 and Figs. 3 to 5 show that the CH_4 expired by dry cow 831 declined rapidly after feeding. This decline is shown in more spectacular form in Fig. 6 on 831 as well as on several lactating cows. (See also Table 4). Methane excretion by cow 831 on the maintenance

TABLE 4.—RESPIRATION EXPERIMENTS WITH LACTATING COWS.

Hrs.	Min.	Resp. Rate R.P.M.	CH₄ %	CO₂ Increment %	O₂ Deficit %	Apparent R. Q.	Corrected R. Q.*	CH₄ Lit./hr.	CH₄ Calories per hr.	Total Heat Cal./hr.
Cow 428, 2/7/36, 128 days of lactation, weight 845 pounds										
0	15	21	0.333	2.859	2.832	1.045	0.791	11.85	112.94	508.75
1	55	19	0.294	2.699	2.621	1.030	0.742	10.99	104.75	494.47
Cow 427, 2/15/36, 13 days of lactation, weight 1100 pounds										
14	30	18	0.176	2.716	3.547	0.766	0.706	4.56	43.46	437.81
2	30	18	0.253	3.240	3.122	1.038	0.859	7.57	72.15	471.67
4	30	20	0.354	2.841	2.945	0.965	0.698	12.70	121.04	527.75
Cow 427, 2/18/36, 16 days of lactation, weight 1100 pounds										
14	30	15	0.218	3.100	3.305	0.938	0.862	4.79	45.65	361.42
0	10	15	0.363	3.438	3.052	1.126	0.903	11.99	114.28	508.84
4		18	0.273	2.943	3.014	0.976	0.774	9.43	89.88	522.79
6	30	16	0.279	3.212	3.384	0.949	0.776	7.06	67.29	426.65
Cow 427, 2/21/36, 19 days of lactation, weight 1100 pounds										
1	--	16	0.378	3.530	3.267	1.081	0.852	11.58	110.37	505.18
3	--	22	0.374	2.826	2.698	1.047	0.733	14.53	138.49	529.16
5	--	33	0.150	2.058	2.021	1.018	0.855	8.13	77.49	552.78
Cow 427, 2/22/36, 20 days of lactation, weight 1100 pounds										
7	--	27	0.151	2.232	2.661	0.839	0.725	6.53	62.24	558.33
9	--	22	0.170	2.618	2.939	0.891	0.792	5.58	53.18	473.90
12	--	22	0.161	2.551	2.982	0.855	0.780	4.33	41.27	390.76
Cow 831, 6/12/36, 18 days of lactation, weight 1081 pounds										
5	--	31	0.262	2.685	2.563	1.048	0.823	11.88	113.23	586.75
7	30	31	0.224	2.654	2.438	1.089	0.909	9.08	86.54	498.88
Cow 427, 6/12/36, 130 days of lactation, weight 1073 pounds										
6	--	33	0.260	2.607	2.206	1.186	0.929	12.90	122.95	552.41
9	30	31	0.172	1.992	1.673	1.191	1.022	11.68	111.32	574.27
Cow 831, 6/13/36, 19 days of lactation, weight 1081 pounds										
3	--	29	0.233	2.820	2.707	1.044	0.846	8.67	82.63	508.63
Cow 427, 6/13/36, 131 days of lactation, weight 1073 pounds										
4	--	30	0.274	2.400	2.105	1.142	0.850	10.27	97.88	398.37
Cow 831, 6/15/36, 21 days of lactation, weight 1081 pounds										
4	30	25	0.174	2.592	2.492	1.043	0.885	6.18	58.90	447.04
8	--	26	0.172	2.396	2.179	1.100	0.951	6.25	59.57	399.69
10	--	23	0.109	2.453	2.439	1.006	0.934	3.24	30.88	365.79
Cow 427, 6/15/36, 133 days of lactation, weight 1073 pounds										
6	--	26	0.220	2.182	2.000	1.094	0.854	10.04	95.69	460.81
Cow 831, 6/22/36, 28 days of lactation, weight 1081 pounds										
2	--	25	0.335	2.682	2.602	1.030	0.758	15.62	148.87	612.31
3	--	22	0.197	2.637	2.610	1.015	0.839	7.76	73.96	519.16
4	--	22	0.223	2.566	2.462	1.042	0.840	9.02	85.97	502.79
5	--	22	0.279	3.024	2.865	1.058	0.841	9.11	86.83	471.93

TABLE 4.—RESPIRATION EXPERIMENTS WITH LACTATING COWS (Continued).

Time After Feeding		Resp. Rate R.P.M.	CH$_4$ %	CO$_2$ Increment %	O$_2$ Deficit %	Apparent R.Q.	Corrected R.Q.*	CH$_4$ Lit./hr.	CH$_4$ Calories per hr.	Total Heat Cal./hr.
Hrs.	Min.									
6	--	24	0.215	2.604	2.409	1.037	0.890	9.77	93.12	552.64
8	--	21	0.216	2.445	2.396	1.020	0.851	8.25	78.63	462.11
10	20	22	0.195	2.486	2.631	0.945	0.830	8.00	76.25	537.38
12	--	18	0.218	2.533	2.717	0.935	0.820	8.29	79.01	513.36
14	--	18	0.198	2.463	2.712	0.911	0.820	7.56	72.05	511.36
Cow 831, 6/24/36, 30 days of lactation, weight 1081 pounds										
2	--	25	0.419	2.973	2.430	1.254	0.858	16.16	154.02	472.91
4	--	23	0.303	2.655	2.581	1.039	0.767	11.55	110.08	496.72
6	--	27	0.269	2.643	2.563	1.033	0.806	9.52	90.74	457.60
8	--	26	0.239	2.620	2.565	1.024	0.846	8.61	82.06	466.27
10	--	22	0.223	2.739	2.801	0.980	0.850	6.97	66.43	439.67
12	--	20	0.214	2.592	2.940	0.983	0.780	6.84	65.19	472.17
14	--	20	0.252	2.707	2.544	1.065	0.945	7.24	69.00	368.70
Cow 834, 6/29/36, 85 days of lactation, weight 870 pounds										
2	--	50	0.245	1.996	1.410	1.416	1.048	16.04	152.88	465.96
4	--	46	0.182	1.866	1.464	1.275	0.997	12.12	115.52	492.08
6	--	51	0.184	1.724	1.105	1.560	1.204	12.93	123.24	391.97
Cow 834, 6/30/36, 86 days of lactation, weight 870 pounds										
4	--	28	0.253	2.330	1.878	1.241	0.940	13.68	130.38	512.66
6	--	28	0.235	2.188	1.712	1.278	0.984	12.48	118.95	458.99
8	--	24	0.229	2.243	1.929	1.167	0.854	9.64	91.88	409.77
11	--	32	0.206	2.197	1.969	1.116	0.959	7.58	72.24	365.42
13	--	28	0.224	2.228	1.852	1.207	1.046	9.60	91.50	400.69
Cow 834, 7/2/36, 88 days of lactation, weight 857 pounds										
4	--	30	0.264	2.166	1.703	1.272	0.926	13.21	125.90	430.01
6	--	30	0.254	2.255	1.807	1.248	0.946	11.74	111.89	421.40
8	--	27	0.208	2.151	1.892	1.137	0.930	10.57	100.74	485.14
10	--	22	0.222	2.441	2.328	1.049	0.896	8.15	77.68	431.20
12	--	23	0.211	2.278	2.338	0.976	0.848	7.83	74.63	435.70
Cow 829, 7/22/36, 299 days of lactation, weight 1034 pounds										
2	--	41	0.205	2.287	1.777	1.287	1.040	----	----	------
4	--	33	0.189	2.309	1.858	1.243	1.016			
7	--	33	0.185	2.330	1.967	1.185	0.993	8.33	79.39	447.17
9	25	40	0.119	1.785	1.737	1.028	0.911	6.37	60.71	469.47
11	30	33	0.120	1.959	1.878	1.043	0.908	5.30	50.51	418.71
13	30	32	0.118	2.184	2.343	0.932	0.867	6.96	66.34	685.93
Cow 428, 7/22/36, 293 days of lactation, weight 900 pounds										
1	--	30	0.259	2.355	1.789	1.316	0.885	----	------	------
3	--	34	0.206	1.893	1.454	1.302	0.980			
5	--	37	0.128	1.851	1.679	1.102	0.934	4.13	39.36	273.11
8	30	37	0.123	1.702	1.546	1.101	0.951	6.32	60.24	401.10
10	20	33	0.120	1.832	1.732	1.058	0.947	5.10	48.61	371.33
12	30	33	0.101	1.630	1.669	0.977	0.892	4.62	44.03	383.23
Cow 829, 7/20/3 , 297 days of lactation, weight 1034 pounds										
4	--	40	0.204	2.424	2.443	0.992	0.806	10.37	98.84	625.16
Cow 428, 7/20/36, 291 days of lactation, weight 900 pounds										
6	--	32	0.167	1.738	1.981	0.877	0.697	8.66	82.54	517.33

*Assuming the same CO$_2$ to combustible gas ratio with time after feeding as found for cow 831 on alfalfa hay and grain feeding.
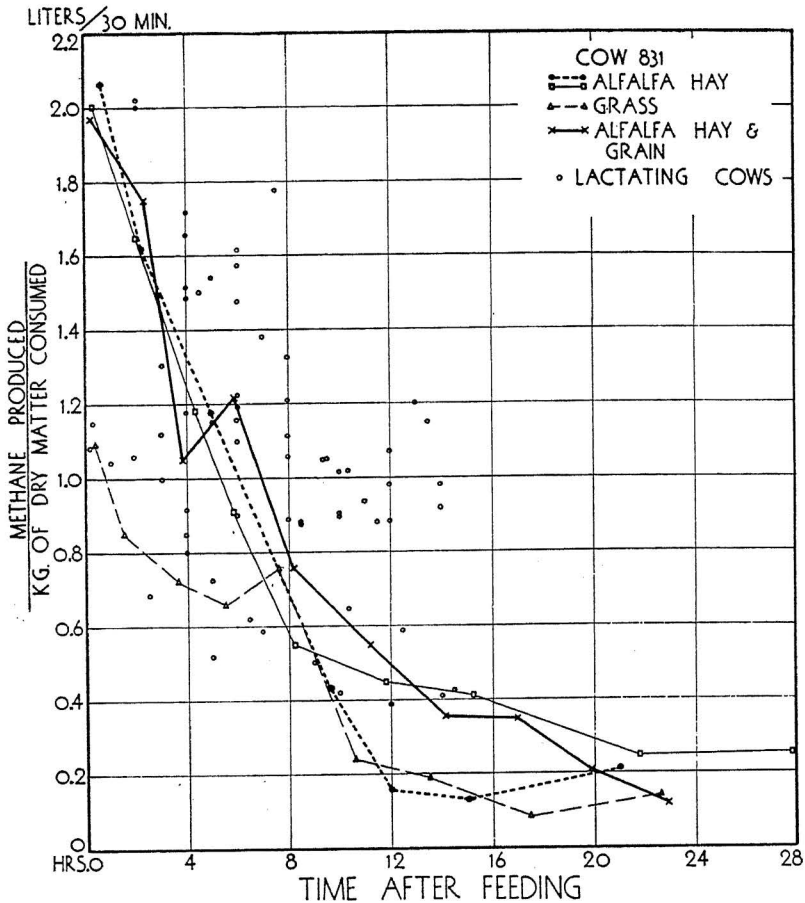
Fig. 6. CH₄ excretion (by mask) per kilo dry weight consumed plotted against time after feeding.

hay ration is seen in Fig. 6 to decline from an initial 2 liters per 30-minutes per Kg. dry matter to 0.2 liter 16 to 20 hours thereafter.

The data on CH_4 excretion and on the ratio CO_2 to CH_4 in the rumen permit correcting the R. Q. for fermentation CO_2, assuming of course that CH_4 and CO_2 diffuse out from the rumen at the same rate. Incidentally, there is an urgent need for investigating the problem of relative passage of CH_4 and CO_2 from the rumen to the outside by way of the lungs. The volume of fermentation CO_2 at a given time was computed by multiplying the CH_4 excreted by the CO_2 to CH_4 ratio in the rumen at the same time. The tissue-metabolism CO_2 was derived by deducting the fermentation CO_2 thus computed from the

TABLE 5.—ENERGY LOSSES IN FERMENTATION GASES, AND RESPIRATORY QUOTIENTS CORRECTED FOR FERMENTATION CO_2 COW 831, WEIGHT 906 POUNDS.*

| Time After Feeding | | Expired CH_4 lit./30 Min. | CO_2 Combustible Gas | Computed Ferm. CO_2 lit./30 Min. | Apparent R. Q. | True R. Q. | R. Q. Difference | Calories equivalent of Fermentation Gases† | |
Hr.	Min.							CH_4	CO_2
			Grass—4485.6 grams dry matter consumed§						
1	25	4.9	3.35	16.1	1.573	1.117	0.456	46.4	68.9
3	30	4.1	3.78	15.6	1.510	1.045	0.465	39.2	67.1
5	35	3.2	2.57	8.4	1.147	0.894	0.253	30.8	35.8
7	30	3.0	2.15	6.4	1.101	0.923	0.178	28.0	27.3
10	34	3.4	2.04	7.0	1.002	0.819	0.183	32.7	30.2
13	35	1.1	2.06	2.2	0.904	0.825	0.079	10.2	9.5
17	30	0.8	1.48	1.3	0.852	0.812	0.040	8.3	5.6
		0.4	0.95	0.4	0.766	0.755	0.011	3.6	1.5
22	32	0.6	1.10	0.7	0.820	0.796	0.024	6.2	3.0
			Alfalfa Hay—2058.4 grams dry matter consumed§						
3	35	4.2	2.52	10.7	1.436	1.047	0.389	40.2	45.8
4	15	3.3	2.20	7.4	1.113	0.872	0.241	31.7	31.6
9	55	2.4	2.08	5.0	1.117	0.953	0.164	22.9	21.6
12	35	0.9	1.65	1.5	0.940	0.890	0.050	8.4	6.4
15	--	0.6	1.16	0.4	0.844	0.831	0.020	3.2	1.7
	--	0.3	0.86	0.3	0.856	0.847	0.009	2.7	1.1
21	--	0.4	0.60‡	0.3	0.932	0.923	0.009	4.1	1.1
			Alfalfa Hay and Grain Mixture—1808 gms. hay, 798.5 gms. grain dry matter§						
2	15	5.2	1.82	9.4	1.268	0.987	0.281	48.8	40.2
3	20	4.6	2.20	10.0	1.298	0.952	0.346	43.3	43.1
5	55	2.8	2.25	6.2	1.139	0.925	0.214	26.2	26.6
8	55	3.2	2.13	6.8	1.279	1.021	0.258	30.3	29.2
11	10	2.0	1.82	3.6	1.076	0.961	0.115	18.8	15.4
14	12	1.4	1.48	2.2	0.922	0.850	0.072	13.7	9.2
17	8	1.0	1.20	1.2	0.932	0.895	0.037	9.1	4.9
	--	0.9	0.78	0.7	0.935	0.905	0.030	8.8	3.1
19	52	0.6	0.58	0.3	0.867	0.851	0.016	5.4	1.4
22	51	0.4	0.48	0.2	0.846	0.839	0.007	3.6	0.8

*As computed from data in Table 3.
†CH_4 9.473 Cal./liter; CO_2, 4.286 Cal./liter.
‡Assumed values at 21 hours.
§Dry matter consumption computed as follows: Grass—30% dry matter; Alfalfa Hay—90.4% dry matter; Grain Mixture—88.5% dry matter.
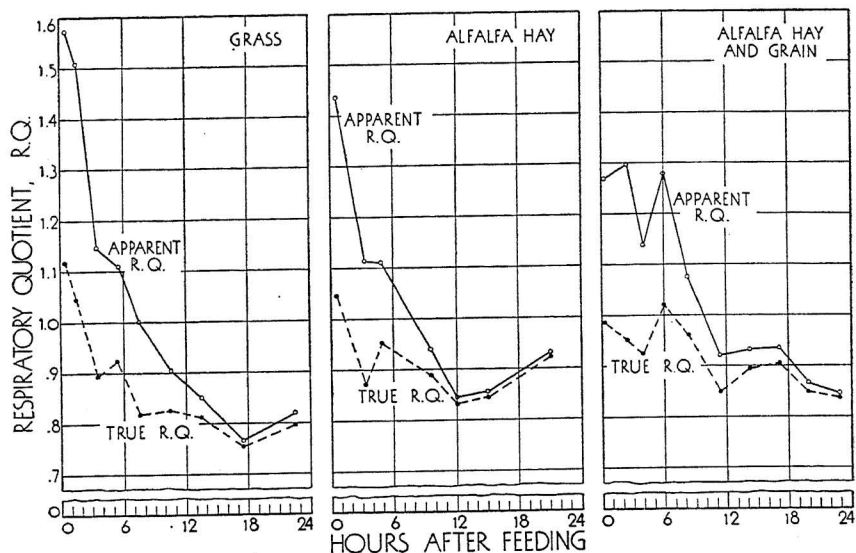
Fig. 7. Time curves of apparent and "true" respiratory quotients.

total CO_2 expired. Table 5 and Fig. 7 present the apparent and the corrected R. Q. computed by this method. Depending on the nature of the feed and on time after feeding, the apparent R. Q. was reduced from 0.007 to 0.465. It is interesting to note that the R. Q. after grass feeding at a 2-maintenance level decreased more rapidly than after a 1-maintenance level of hay and grain. Grass appears to be not only more rapidly digested, but its digestion involves less CH_4 formation. The meaning of the relatively low CH_4 and high CO_2 on the grass diet is not clear to us.

Feed energy losses associated with fermentation CH_4 and CO_2 are shown in Table 5. In these computations it was assumed that the fuel value of CH_4 was 9.473 Calories per liter; energy equivalence of CO_2, 4.286 Calories per liter (energy liberated in complete oxidation of carbon in charcoal to CO_2); dry matter in hay and grass, 4.5 Calories per gram, and in grain 5.5 Calories per gram. These losses were as follows: maintenance ration of alfalfa hay, 16.5 per cent of the energy intake or about 25 per cent of the maintenance requirement; alfalfa hay and grain (slightly over maintenance), 14.6 per cent of the energy intake, or about 30 per cent of the maintenance requirement; for grass (about twice maintenance intake of dry matter) 12 per cent of the energy intake, or about 40 per cent of the maintenance requirement.

One of the principal aims of this investigation was to formulate correction factors for the accumulation of CH_4 in spirometers during

TABLE 6a.—GAS VOLUMES IN DIFFERENT PARTS OF DIGESTIVE TRACTS OF SLAUGHTERED ANIMALS.

Animal	Weight Pounds	Rumen Liters	Abomasum Liters	Small Intestine Liters	Large Intestine Liters	Total Liters	% of Total in Rumen
Sheep 6	103.5	7.276	0.071	0.015	0.132	7.494	97.09
Sheep 7	100.0	1.596	0.019	0.010	0.067	1.692	94.33
Sheep 62	122.0	2.633	0.063	No gas	0.055	2.751	95.71
Sheep 86	112.0	2.280	0.034	0.010	0.240	2.564	88.92
Sheep 111	133.0	2.446	0.049	0.027	0.143	2.665	91.78
Sheep 31	110.0	1.223	No gas	0.007	0.055	1.285	95.18
Sheep 55	137.0	1.327	No gas	0.030	0.037	1.394	95.19
Sheep 986	112.0	3.628	No gas	0.006	0.100	3.734	97.16
Hereford Heifer	-----	4.726	No gas	Few bubbles	0.785	5.511	85.76
Goat 647	63.5	0.290	No gas	No gas	No gas	0.290	100.00
Goat 591	79.5	0.454	No gas	Few bubbles	0.076	0.530	85.66
Goat 639	39.0	0.578	0.021	Few bubbles	0.010	0.609	94.91
Goat 619	67.0	0.677	0.006	Few bubbles	0.225	0.908	74.56
Sheep 154	135.0	1.182	No gas	Few bubbles	0.240	1.422	83.12
Goat 625	64.5	0.848	Few bubbles	Few bubbles	Few bubbles	0.848	100.00
Sheep 17	101.0	0.870	Few bubbles	Few bubbles	0.124	0.994	87.53
Goat 825	41.0	0.879	Few bubbles	Few bubbles	Few bubbles	0.879	100.00
Goat 601	35.5	1.500	0.010	Few bubbles	0.025	1.535	97.72
Goat 627	73.0	1.417	Few bubbles	Few bubbles	0.214	1.631	86.88
Goat 633	67.5	0.497	Few bubbles	Few bubbles	Few bubbles	0.497	100.00
							% of Total in Large Intestine
Horse 24	----	-----	-----	0.642	11.194	11.836	94.57

TABLE 6b.—RUMEN GAS COMPOSITION IN GOATS KILLED AT DIFFERENT TIMES AFTER FEEDING.

Goat No.	Time After Feeding Alfalfa Hay and Grain, Hours	Percentages of					Ratios			
		CO_2	CH_4	H_2	O_2	N_2	$\dfrac{CO_2}{\text{Combustible Gas}}$	$\dfrac{N_2}{O_2}$	$\dfrac{CH_4}{H_2}$	$\dfrac{CO_2}{O_2}$
601	1.0	73.91	25.19	1.98			2.86		12.7	
639	2.5	68.23	26.77		1.90	3.10	2.55	1.6		35.9
825	4.0	64.75	31.95	0.98	3.63	----	2.00	---	----	17.8
627	12.0	65.06	32.38	1.25	1.13	----	1.98	---	32.6	57.6
619	12.0	40.20	47.54	2.74	2.43	7.10	0.82	2.9	20.9	16.5
591	24.0	21.62	28.85	2.59	9.16	37.78	0.71	4.1	17.4	2.4
633	48.0	8.89	16.68	1.30	9.71	63.41	0.50	6.5	11.1	0.9
625	48.0	20.54	29.51	3.96	12.30	33.68	0.64	2.7	12.8	1.7
647	72.0	9.47	14.62	1.75	12.13	62.02	0.60	5.1	7.5	0.8
660	72.0	10.76	12.24	----	7.20	69.79	0.88	9.7	8.4	1.5

TABLE 6c.—RUMEN CO_2/COMBUSTIBLE GAS RATIOS IN SLAUGHTERED ANIMALS.

Animal	Feed*	Time After Feeding Hours	CO_2/ Combustible Gas Ratio
Holstein Cow	A	4.0	2.40
Hereford Calf	Unknown	Unknown	2.32
Sheep No. 111	B	About 12 hours	2.17
Aberdeen Angus Calf	Unknown	Unknown	1.81
Sheep No. 6	B	About 12 hours	1.74
Sheep No 37	B	About 12 hours	1.43
Sheep No. 86	B	About 12 hours	1.41
Aberdeen Angus Cow	Unknown	Unknown	1.35
Sheep No. 986	B	About 12 hours	1.33
Sheep No. 62	B	About 12 hours	1.32
Hereford Cow	Unknown	Unknown	1.15
Sheep No. 31	B	About 12 hours	1.12
Sheep No. 55	B	About 12 hours	1.01
Sheep No. 7	B	About 12 hours	0.86
Hereford Calf	Unknown	Unknown	0.81
Sheep No. 154	B	About 12 hours	0.65

*Feed A—Alfalfa Hay, Silage, and Grain Mixture.
Feed B—Alfalfa Hay, and Straw.

TABLE 6d.—COMPOSITION OF LARGE INTESTINE GAS IN SLAUGHTERED ANIMALS.

| Animal | Percentages of | | | | | Ratios | | | |
	CO_2	CH_4	H_2	O_2	N_2	CO_2 : Combustible Gas	$\dfrac{N_2}{O_2}$	$\dfrac{CH_4}{H_2}$	$\dfrac{CO_2}{O_2}$
Goat No. 639_____	33.11	46.28	2.59	1.28	16.74	0.69	13.1	17.9	25.9
Goat No. 619_____	21.49	60.18	2.81	1.78	13.75	0.34	7.7	21.4	12.1
Goat No. 591_____	25.16	43.02	3.15	2.59	26.09	0.56	10.1	13.7	9.7
Goat No. 660_____	18.41	28.74	1.75	----	51.10	0.62	----	16.4	----
Sheep No. 62_____	15.13	75.05	----	(Not Determined)_____		0.20	----	----	----
Sheep No. 96_____	38.76	53.75	----	(Not Determined)_____		0.72	----	----	----
Shepp No. 55_____	25.57	54.69	1.78	(Not Determined)_____		0.45	----	30.7	----
Aberdeen Angus Cow___	10.20	45.41	3.76	(Not Determined)_____		0.20	----	12.1	----
Sheep No. 6_____	21.13	59.46	----	(Not Determined)_____		0.36	----	----	----
Sheep No. 31_____	30.46	53.30	----	(Not Determined)_____		0.57	----	----	----
Hereford Calf_____	30.79	35.06	----	(Not Determined)_____		0.88	----	----	----
Sheep No. 86_____	14.10	55.45	----	(Not Determined)_____		0.25	----	----	----
Horse No. 24_____	89.43	7.97	----	(Not Determined)_____		11.22	----	----	----

TABLE 6e.—CO_2/COMBUSTIBLE GAS RATIOS IN ABOMASUM AND SMALL INTESTINE OF SLAUGHTERED ANIMALS.

| Animal | Organ | Percentages of | | | Ratios | |
		CO_2	CH_4	H_2	$\dfrac{CO_2}{\text{Combustible Gas}}$	$\dfrac{CH_4}{H_2}$
Sheep No. 6_____	Small Intestine_____	3.85	58.08	----	0.07	----
Sheep No. 111_____	Small Intestine_____	12.36	40.80	----	0.30	----
Sheep No. 55_____	Small Intestine_____	6.42	16.49	1.50	0.36	4.3
Sheep No. 86_____	Small Intestine_____	6.61	11.41	----	0.58	----
Sheep No. 111_____	Abomasum_____	18.53	46.97	1.44	0.39	12.9
Sheep No. 86_____	Abomasum_____	33.50	53.09	----	0.63	----
Sheep No. 62_____	Abomasum_____	37.74	55.15	----	0.68	----

TABLE 7.—COMPARISON OF APPARENT AND "TRUE" (CORRECTED FOR CH₄) OXYGEN CONSUMPTION BY THE BENEDICT-COLLINS SPIROMETER METHOD, AND OXYGEN CONSUMPTION BY THE NEW OPEN CIRCUIT METHOD.

		Spirometer				Open-Circuit		
Hours After Feeding		Apparent O₂ Liters per 15 minutes	CH₄ Liters per 15 minutes	"True" O₂ Liters per 15 minutes	Ratio Apparent O₂ / True O₂ %	Hours After Feeding		O₂ Liters per 15 minutes	CH₄ Liters per 15 minutes
Hrs.	Min.					Hrs.	Min.		
						2	25	13.470	2.182
1	20	12.540	1.741	14.085	112.3	2	0	15.660	1.795
2	50	13.725	1.133	14.865	108.3	4	25	14.265	1.285
4	50	14.715	1.787	16.365	111.2	5	50	17.505	0.995
6	40	13.695	0.483	14.160	103.4	9	15	17.955	0.602
10	5	15.195	0.250	15.435	101.6	11	50	14.775	0.495
12	40	12.465	1.067	13.530	108.5	15	15	12.930	0.457
16	5	11.775	0.250	12.015	102.0	21	50	11.910	0.245
22	35	9.855	0.411	10.260	104.1	27	50	11.175	0.287
28	30	12.315	0.141	12.465	101.2	33	40	10.440	0.285
34	40	14.565	0.226	14.790	101.5	46	0	11.655	0.282
46	40	11.940	0.192	12.120	101.5	51	0	11.355	0.200
52	45	11.130	0.160	11.280	101.3	58	0	16.005	0.057
58	40	15.990	0.154	16.140	100.9	69	30	12.810	0.000
70	40	13.650	0.110	13.755	100.7	76	15	12.780	0.152

measurements of oxygen consumption with the Benedict-Collins clinical method. The results for a 70-hour fasting experiment are given in Table 7 and Fig. 8. Time curves following feeding are shown for CH_4 accumulated in the spirometer, apparent consumed O_2, "true" consumed O_2 (corrected for the CH_4), and consumed O_2 as determined in the same experiment with the open-circuit method. The error for CH_4 accumulation is appreciable during the early hours after feeding, and, of course, declines along with CH_4 decline with time after feeding. The fluctuation of the oxygen intake after about 30 hours of fasting (Fig. 8) was probably due to the fact that the animal had coccidiosis and became irritable and passed some blood during prolonged fasting.
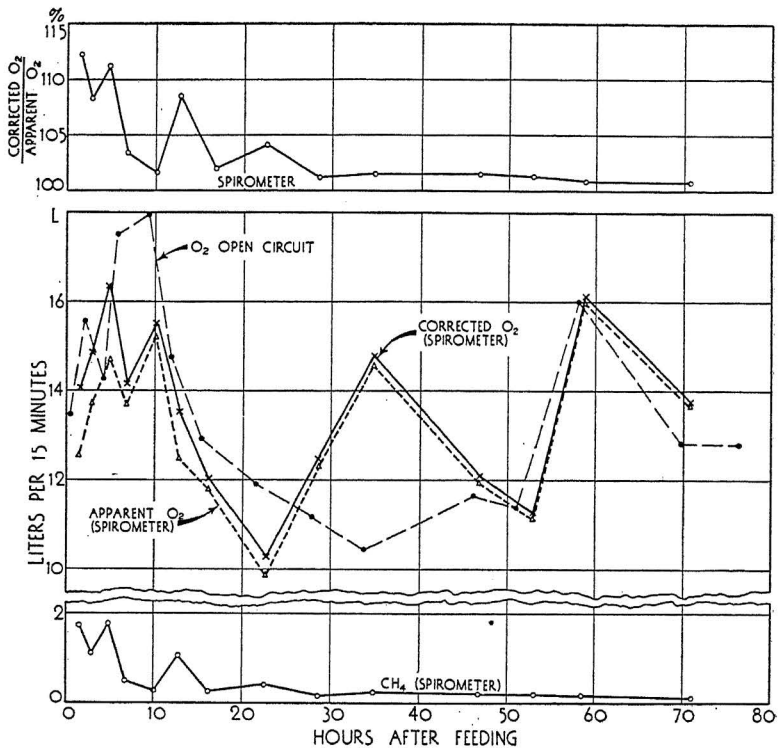
Fig. 8. Time curves of CH_4 accumulation in spirometer by the Benedict-Collins method; also apparent and "true" O_2 consumption by this method; and O_2 consumption by the open-circuit method.

Finally we like to mention an interesting by-product of this investigation exhibited in Fig. 9. If the "true" respiratory quotient of lactating dairy cattle (average corrected R. Q. taken from several respi-
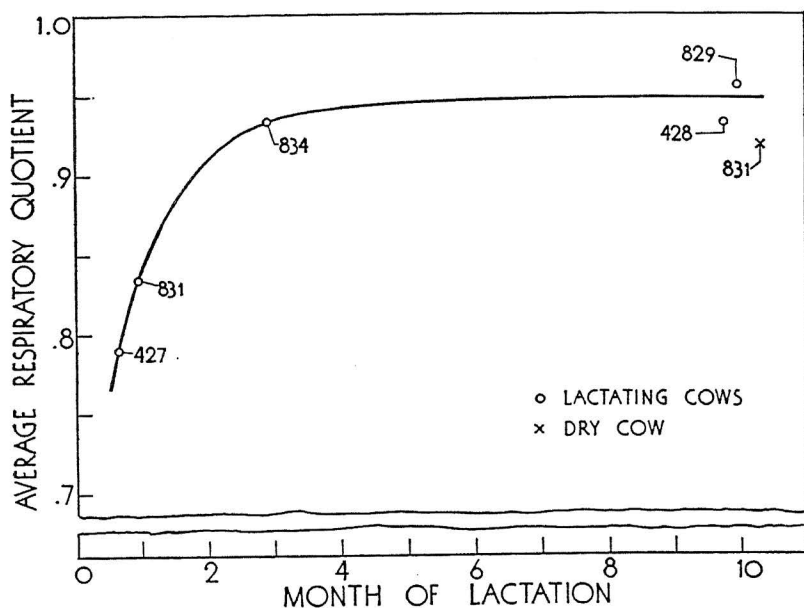
Fig. 9.   Rise of repiratory quotient with advance of the stage of lactation.

ration periods covering a 12 to 14 hour interval between milkings) is plotted against month of lactation, it rises from less than 0.8 in the early period of lactation, to over 0.9 by the 10th month of lactation. The rise appears to be exponential; that is, the successive increases are successively smaller approaching an upper limit of perhaps 1.0. The animals did not fast; they were in normal nutritive condition between milkings. What is the possible explanation of the early low R. Q. and the subsequent rise? Does it reflect the early carbohydrate drain for milk production, or the early inability of the organism to maintain a "normal" blood sugar in the face of the heavy demand by the mammary gland? This suggests the need for establishing time curves during the lactation period for blood sugar level, alkaline reserve, and possible presence of acetone bodies.

B.   **Slaughtered Animals**: In living animals, only rumen gas could be conveniently secured for analysis. It was necessary, however, to determine not only the composition but also gas volume in the rumen, and not only the rumen gas, but also the volumes and composition of the gases in other parts of the digestive tract. Slaughtered animals were used for these determinations with results shown in Table 6a to 6c.

Table 6a shows that the rumen contains from 75 to 100% of the alimentary gas. In animals slaughtered within 24 hours after feeding,

96% of the gas was found in the rumen. 100% of the gas was found in the rumen in goats slaughtered 48 to 72 hours after feeding. There was almost no gas in the intestine in fasted animals, due perhaps to the dehydrated condition of the intestinal contents. The rumen, on the other hand, retains liquid during fasting, which may explain the constancy of rumen gas volume. Table 6a, listing the distribution of gas in the various parts of the digestive tract, is supplemented by Table 8 listing the corresponding distribution of fill in the various parts of the tract.

The ratios of rumen CO_2 to CH_4 (or rather to combustible gases) at different times after feeding are shown in Table 6b. The time course of decline after feeding in the slaughtered goats is not very much different from the time course found in the living cow (Table 1). By 24 hours after feeding, the rumen CO_2 to CH_4 ratio had declined to a value approximating that found in the intestine, shown in Table 6d. Table 6b on animals slaughtered at known times after feeding is supplemented by Table 6c on animals slaughtered at unknown times after feeding.

As in the case of living animals, Table 6b shows that the time course of the CO_2 to CH_4 ratio in slaughtered animals reflects not changes in CH_4 but in CO_2 percentages. The relation between N_2 and O_2 is also similar to that in living animals.

A striking feature about the alimentary gas, is the relatively low CO_2 to CH_4 ratio (about 0.5) in the intestine as compared to that found in the rumen. Markoff attributed low ratios in ox rectum gases to greater diffusibility of CO_2 than CH_4 from the intestine to the blood. It does not seem, however, that this completely explains the low intestinal ratios. Probably differences in bacterial flora and type of substrate fermented are also factors which bring about different relationships between carbon dioxide and combustible gases. This explanation is supported by the fact that rumen ratios during fasting also reach values given by intestinal gases. It does not seem that the diffusion of carbon dioxide and combustible gases from the rumen would change enough in an interval of 24 hours of fasting to account for the low ratios shown in Tables 1, 2, 6b and 6c. Furthermore, an extremely high ratio of 11.22 was found for the large intestine gases of a horse (Table 6d). It is improbable that differences in diffusibility from the intestines of the ruminant and the horse would be great enough to account for such differences in ratios.

The comparative study of digestive-tract gases proved to be entirely worthwhile. Time changes in the composition of rumen gas found in living animals were substantiated by the data on slaughtered animals. The volume distribution and the composition of gases in

TABLE 8.—ALIMENTARY FILL IN SLAUGHTERED ANIMALS

Date	Animal	Live Weight Kg.	Time After Feeding Hr. Min.	Rumen + Reticulum Kg.	Omasum Kg.	Abomasum Kg.	Small Intes. Kg.	Large Intes. Kg.	Total Wt. Alim. con. Kg.	Percent of Live Weight as Fill	Percent of Total in Rumen + Ret.
										%	%
12–30–36	Sheep 6	46.9	About 12 hr.	7.920	0.416	0.657	0.962	1.371	11.326	24.15	69.93
12–31–36	Sheep 7	45.4	About 12 hr.	3.083	0.165	0.590	0.503	0.974	5.315	17.07	58.01
1– 1–37	Sheep 62	55.0	About 12 hr.	9.063	0.105	0.273	0.486	1.145	11.072	20.13	81.86
1– 1–37	Sheep 86	50.8	About 12 hr.	7.431	0.284	0.384	0.627	1.507	10.233	20.14	72.62
1– 2–37	Sheep 63	----	About 12 hr.	5.257	0.223	0.228	0.300	0.599	6.607	-----	79.57
1– 2–37	Sheep 37	----	About 12 hr.	5.811	0.300	0.274	0.165	0.855	7.405	-----	78.47
1– 7–37	Sheep 111	60.3	About 12 hr.	8.563	0.304	0.575	0.630	1.543	11.615	19.26	73.72
1– 8–37	Sheep 31	49.9	About 12 hr.	6.070	0.202	0.689	0.645	1.242	8.848	17.73	68.60
1– 8–37	Sheep 55	62.1	About 12 hr.	9.101	0.323	0.649	0.980	1.062	12.115	19.51	75.12
1– 9–37	Sheep 986	50.8	About 12 hr.	9.141	-----	0.941	0.604	1.210	11.896	23.42	76.84
1–12–37	Herf. Heifer	----	Unknown	28.978	1.688	2.480	1.431	2.874	37.451	-----	77.73
1–16–37	Sheep 154	61.2	About 12 hr.	10.249	0.135	0.926	0.833	1.111	13.254	21.66	77.33
1–16–37	Sheep 17	45.8	About 12 hr.	6.749	0.250	0.433	1.187	1.874	10.493	22.91	64.32
1–16–37	Sheep 6	52.6	About 12 hr.	7.112	0.295	0.552	0.882	1.694	10.535	20.03	67.51
1–18–37	Sheep 655	61.2	About 12 hr.	11.119	0.238	0.419	1.046	1.299	14.121	23.07	78.74
1–18–37	Sheep 64	56.7	About 12 hr.	10.603	0.226	0.580	1.043	1.204	13.656	24.08	77.64
1–18–37	Sheep 98	50.1	About 12 hr.	8.297	0.229	0.679	0.569	1.005	10.779	21.51	76.97
1–23–37	Herf. Calf	----	Unknown	44.778	2.595	0.788	1.995	2.773	52.929	-----	84.60
1–28–37	Horse 24	----	Unknown	-----	-----	-----	5.300	47.269	52.569	-----	-----
2–15–37	Goat 591†	36.1	24 0	1.746	0.111	0.052	0.123	0.274	2.306	6.39	75.72
2–11–37	Goat 647†	28.8	72 0	2.096	0.096	0.060	0.112	0.228	2.592	9.00	80.86
2–12–37	Goat 625†	29.3	48 0	1.553	0.069	0.014	0.107	0.069	1.812	6.18	85.71
2–13–37	Goat 633†	30.6	48 0	2.004	0.054	0.067	0.185	0.142	2.452	8.01	81.73
2–15–37	Goat 600	27.2	About 12 hr.	4.115	0.067	0.260	0.208	0.176	4.826	17.74	85.27
2–16–37	Goat 825*	18.6	4 0	3.206	0.012	0.167	0.298	0.310	3.993	21.47	80.29
2–18–37	Goat 639*	17.7	2 30	1.658	0.038	0.057	0.077	0.207	2.037	11.51	81.39
2–20–37	Goat 601*	16.1	1 0	1.964	0.019	0.123	0.221	0.219	2.546	15.81	77.14
2–22–37	Goat 619	30.4	12 0	3.567	-----	0.121	0.498	0.829	5.105	16.49	71.13
2–23–37	Goat 627	33.1	12 0	3.308	0.012	0.335	0.466	0.817	4.938	14.92	66.99

*Half-grown goats.   †Pregnant,

various regions of the digestive tract seem to indicate that intestinal fermentation gases make up a very small part of the over-all fermentation gas production. However, attention is called to the need of a study of the relative diffusiblity of CO_2 and CH_4 in various parts of the tract and to the relative amounts of CO_2 and of CH_4 which escape by the principal exits.

V. SUMMARY AND CONCLUSIONS

The principal contributions of this bulletin are: 1. Method for securing from farm animals for analysis directly expired air which is uncontaminated by respiration chamber or outdoor air. 2. This method enables evaluation of O_2, CO_2 and CH_4 for short periods. With the aid of this method on expired air, accurate time curves were charted for CO_2 and CH_4 excretion and O_2 consumption following feeding. 3. Paralleling the time curves on expired air, time curves were secured following feeding on the composition of rumen gas of living animals. 4. The volume distribution and the composition of gases in various regions of the digestive tracts of slaughtered animals were also determined. 5. Assuming that CO_2 and CH_4 diffuse out from the rumen at equal rates, the total CO_2 expired was corrected for fermentation CO_2, leading to computations of "true" R. Q.s as contrasted to "apparent" R. Q.'s formerly published.

The above investigation led to the following conclusions: 1. The rumen CO_2 to CH_4 ratio is not a constant, but declines in a typical manner with time after feeding. The correction of the respiratory quotient for fermentation CO_2 is thus likewise not a constant but depends on time after feeding. 2. The feed energy losses by fermentation as determined from the energy equivalent of CO_2, and CH_4, were estimated to be about 25% of the maintenance requirement of the animal, while on a maintenance feed level. The absolute losses decline with time after feeding. 3. A small amount of oxygen is taken into the rumen during eating, and is used up immediately, probably in aerobic processes. 4. Rumination does not cause a measurable increase in rumen CO_2 or O_2. 5. More rapid diffusion of CO_2 into the blood does not completely explain the low intestinal CO_2 to CH_4 ratio. Like ratios are also found in the rumen during fasting, and a very high ratio was found in the horse large intestine. 6. The increase in rumen N_2 and O_2 with time after feeding can not be adequately explained on the basis of the diffusion of these gases into the rumen.

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