FACTORS INFLUENCING PULMONARY METHANE EXCRETION IN MAN

An Indirect Method of Studying the In Situ Metabolism of the Methane-Producing Colonic Bacteria*

By JOHN H. BOND, JR., M.D., ROLF R. ENGEL, M.D., and MICHAEL D. LEVITT, M.D.

(*From the Departments of Medicine and Pediatrics, University of Minnesota Hospital, Minneapolis, Minnesota 55455*)

(Received for publication 29 October 1970)

It is becoming increasingly apparent that the metabolic activity of the normal intestinal flora may exert a profound influence on the physiology of their host (1). There is, however, only limited information concerning the metabolism of these bacteria, and this information has been derived primarily from studies carried out with isolated species in culture media. The results of such studies may not accurately reflect the *in situ* behavior of these organisms within the complex environment of the bowel.

In the present investigation, we studied the production of a bacterial metabolite, methane (CH_4), in man. Measurement of the pulmonary excretion rate of methane appeared to offer a unique opportunity to study the *in situ* metabolism of a group of intestinal organisms since it is commonly assumed that (*a*) all CH_4 excreted by man is derived from the metabolism of intestinal bacteria, and (*b*) there is no appreciable utilization of CH_4 by man; thus, all CH_4 absorbed from the colon will be quantitatively eliminated by the lungs.

In this investigation we first attempted to demonstrate that pulmonary CH_4 excretion can serve as an accurate indicator of CH_4 production by intestinal bacteria. Subsequent studies were then directed towards elucidating the factors which influence the rate of production of this bacterial metabolite in the human colon.

Materials and Methods

Subjects and Experimental Animals.—The pulmonary excretion rate of CH_4 was studied in 20 infants ranging in age from 2 hr to 5 months and in 22 normal adults. The CH_4 concentration of end-expiratory air was measured in 280 adults and 40 children. The adults consisted of 180 normal subjects and 100 patients hospitalized on a general medical ward. The children consisted of 25 healthy subjects and 15 children hospitalized with various cardiac defects.

* Supported by U.S. Public Health Service Grants Rol HD04487, AM13309, 5 FO3 AM42999, and AM05025.

In addition to this sampling, the CH_4 concentration of expired air was measured in the following groups of subjects: (*a*) 25 families (parents plus children) randomly selected from the community; (*b*) 35 elderly inhabitants of a veterans' home; (*c*) 31 patients living in two separate houses at a school for the mentally retarded and 17 employees of this school; and (*d*) 36 sets of twins (11 identical and 25 fraternal). The mentally retarded subjects represented a heterogeneous group of conditions including birth trauma, congenital defects, and mongolism. The site and rate of intestinal CH_4 production were studied in 14 healthy, fasting adults.

Three, 100 g, germfree rats[1] were studied for CH_4 excretion before and after contamination with fecal bacteria.

Measurement of Rate of Methane Excretion.—The rate of pulmonary methane excretion in adults was measured by having the subject rebreath for 2–6 hr into a closed system somewhat similar to that described by Coburn et al. (2). Briefly, the subject's head was enclosed in a polyvinyl hood which was sealed at the neck with a rubber diaphragm. The gas in the hood was circulated via a pump through a CO_2 absorber, an ice bath, a spirometer, and then back into the hood. Oxygen was added to the system via a solenoid which was activated by a magnetic switch when the spirometer fell to a certain level. The total volume of gas in the system was measured by injecting a known volume of helium at the start of the study. This system had a small and variable leak which averaged about 5% of the gas volume per hour. This leak rate was determined and corrected for by measurement of the decrease in helium concentration which occurred during the experiment.

Methane excretion in infants was measured using a similar closed system with several modifications. A glass hood was connected to a polyurethane bag[2] which enclosed the entire infant. The spirometer used in the adult collecting system was eliminated and O_2 inflow was regulated by a Model 20 CA Teledyne[3] oxygen analyzer, adjusted to maintain a P_{O_2} of 150 $\pm$ 5 mm Hg. Because ordinary commercial O_2 may contain up to 30 ppm of CH_4, it was necessary to use an ultrapure O_2 supply[4] (no detectable CH_4) in these closed system measurements of CH_4 excretion.

The excretion rate of CH_4 by rats was also determined using a similar closed system technique. The rat was placed in a polyvinyl cylinder. An O_2 reservoir under a 2–3 cm H_2O-positive pressure was connected to the cylinder and O_2 entered the system as CO_2 was absorbed. The gas volume of this system was calculated from the volume of the system (milliliters) when empty minus the weight of the rat in grams.

In each of these closed systems, the quantity of CH_4 excreted per unit of time was determined from the volume of gas in the system and the concentration of CH_4 present in periodically analyzed samples.

Concentration of Methane in End-Expiratory Air.—Breath CH_4 concentration was used as a rough indicator of the pulmonary excretion rate of CH_4. Samples of expired air were collected by having the subject exhale through a plastic mouth-piece connected by a three-way valve to a 50 ml syringe. The valve was manipulated during expiration so as to fill the syringe with the end-expiratory fraction of exhaled air. Before collection, subjects were instructed to breathe normally in order to prevent precollection hyperventillation. The gas samples were analyzed for O_2 as well as CH_4 and only those collections with a P_{O_2} of less than 125 mm Hg were considered adequate samplings of end-expiratory air.

Site and Rate of CH_4 Production in the Bowel.—A constant gas perfusion technique which

[1] Charles River Breeding Laboratories, North Wilmington, Mass.

[2] Winzen Research, Inc., Bloomington, Minn.

[3] Teledyne Analytic Instruments, San Gabriel, Calif.

[4] Air Reduction Company, Inc., Riverton, N. J.

has previously been described (3) for the study of intestinal H_2 production was employed. Briefly, the subjects were intubated with a mercury-weighted, triple-lumen, polyvinyl tube. The tube was passed until the distal opening (distal collecting site) was fluoroscopically located in the terminal ileum. The middle opening (proximal collecting site) and proximal opening (infusion site) of the tube were located 60 and 120 cm proximal to the distal orifice in the proximal ileum and mid-jejunum respectively. Nitrogen (13 studies) or air (1 study), containing 0.5% sulfur hexaflouride (SF_6), was constantly infused into the bowel via the proximal orifice of the tube at a rate of 30 ml/min. SF_6 is a gas that is only minimally absorbed from the bowel and therefore can be used as a dilutional marker for the gas infusate similar to the use of polyethylene glycol for a liquid infusate.

Gas samples were obtained in lubricated syringes from the proximal and distal ileum via the intestinal tube and the rectum via a rectal tube. 2 g of lactose (20 ml of a 10% solution) was then rapidly infused through each lumen of the tube. Constant perfusion of the intestine was continued. Gas was sampled from the proximal ileum, terminal ileum, and rectum at 30, 60, and 90 min after lactose instillation. The rate that CH_4 passed each collection site was calculated using standard equations for constant perfusion techniques (4).

Methane Liberation During Incubation of Fecal Specimens.—20 g of a freshly passed stool specimen from each of eight normal subjects was homogenized in 20 ml of 0.2M phosphate-buffered saline (pH 7.0). 10 ml of this homogenate was placed in a test tube and incubated at 37°C for 1 hr. The evolved gas was collected, the volume measured, and the CH_4 concentration determined.

Analysis of Samples.—The studies reported in this paper were carried out in three laboratories, and therefore several different techniques were used to analyze for CH_4. In all studies, a gas chromatograph equipped with a gas sampling valve and molecular sieve column was used. In the infant studies, nitrogen served as the carrier gas and a hydrogen-flame detector was employed. Breath CH_4 excretion in adults was determined using argon as a carrier gas, and thermal conductivity and hydrogen-flame detectors were connected in series to measure O_2 and CH_4 respectively. Measurement of CH_4, H_2, and SF_6 in the intestinal perfusion and the rat studies employed a thermal conductivity detector.

RESULTS

Site and Rate of CH_4 Production in the Bowel.—During the intestinal perfusion experiments CH_4 was never detected in gas sampled from the mid-jejunum or ileum. In 5 of the 14 subjects, CH_4 was readily detected in gas passed via rectum with the fasting rate of colonic CH_4 production of these 5 subjects averaging 0.45 ± 0.13 (1 SE) ml/min. In the other 9 subjects CH_4 was not detectable (<100 ppm) indicating a CH_4 production of less than 0.003 ml/min. The rate of CH_4 production was not significantly altered by the infusion of lactose into the bowel.

In one CH_4-producing subject, the triple-lumen tube fortuitously passed until the distal collecting site was located in the splenic flexure. Only about 9% of the methane was produced proximal to the splenic flexure while simultaneous measurements of H_2 production indicated that about 57% of the colonic H_2 production occurred proximal to this collecting site.

Bacterial Origin of CH_4 Production.—In an attempt to prove that CH_4 is solely derived from bacterial metabolism, studies were carried out in germfree rats and newborn infants. As shown in Fig. 1, CH_4 excretion was not detected

by rats in the germfree state; however, CH_4 excretion became detectable within 5 days of contamination of these rats with fecal material from a rat previously shown to produce large quantities of CH_4. CH_4 excretion was never detected ($<6 \times 10^{-6}$ ml/min) in infants up to 6 months of age. In contrast, another bacterial product (H_2) was detected within 24 hr of life (5).

Utilization of CH_4.—In order to determine if CH_4 was metabolized by man, the rate of removal from the closed system of exogenously added CH_4 was studied in three nonproducers. In each of these subjects, the concentration of CH_4 and He fell at identical rates indicating no detectable utilization of CH_4.

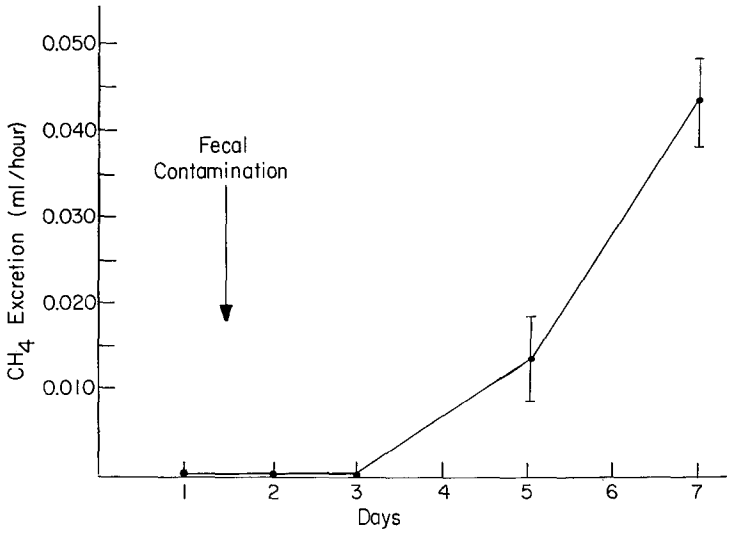

FIG. 1. Methane excretion of three rats in the germfree state and after contamination with fecal material from a CH_4-producing rat.

Pulmonary Excretion Rate of CH_4.—The rate of pulmonary CH_4 excretion of 22 adults is shown in Fig. 2. These studies, like the colonic perfusion experiments, demonstrated that there are large individual differences in the rate of CH_4 excretion, ranging from undetectable ($<5 \times 10^{-6}$ ml/min) up to 0.66 ml/min. In general, subjects excreted little or no CH_4, or relatively large quantities of CH_4. This finding suggested that measurement of the concentration of CH_4 in expired air might serve as a sufficiently accurate indicator of CH_4 excretion for epidemiologic studies.

Measurement of CH_4 Concentration in End-Expiratory Air.—A variety of techniques were tested in an attempt to obtain the most reproducible and accurate measurement of alveolar CH_4 concentration. Because of the low solubility of CH_4 in blood relative to air, transient hyperventilation before the collection of breath samples would rapidly wash out the CH_4 present in the

lungs. Using the technique described in Materials and Methods, the CH_4 concentration in consecutive samples of expired air varied by less than $\pm 20\%$. Fig. 3 shows the relatively good correlation that exists between breath CH_4 concentration and the respiratory excretion rate of CH_4 of 22 subjects.

Lastly, the relationship between the breath CH_4 concentrations and production by fecal homogenates was studied in eight subjects. As shown in Table I, breath CH_4 concentration reflected CH_4 production by the fecal homogenates.

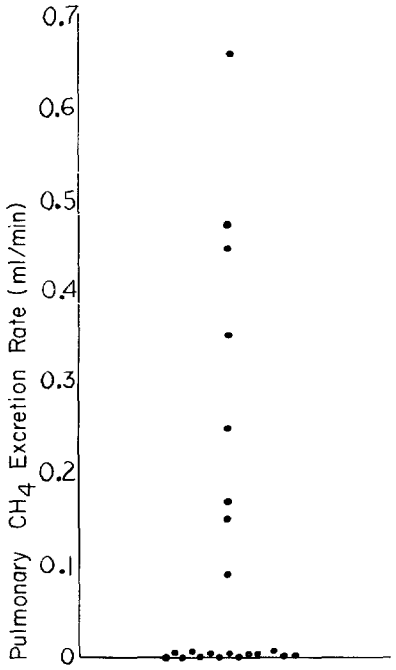

FIG. 2. Pulmonary CH_4 excretion rate of 22 adults.

End-Expiratory CH_4 Concentration in the Adult Population.—The breath CH_4 concentration of 280 adults is shown in Fig. 4. While there is no clear-cut break in the distribution of these results, we divided the population into two groups on the basis of breath CH_4 concentrations. 66.4% of the subjects had a breath CH_4 concentration of less than 1 ppm (mean = 0.122 ppm) above the concentration of CH_4 present in the atmosphere which averages about 1.8 ppm. The remaining 33.6% of the subjects excreted readily measurable quantities of CH_4 ranging from 1 to 70 ppm above atmospheric CH_4 with an average of 14.8 ppm. For the purpose of this study, subjects with breath CH_4 concentrations greater than 1 ppm above atmospheric CH_4 will be arbitrarily designated producers of CH_4 and those with less than 1 ppm, nonproducers.

These 280 subjects included several subgroups of individuals: (*a*) 100 medical students, house staff, and nurses living in an urban area; (*b*) 91 hospitalized patients not on antibiotic therapy; (*c*) 37 hospitalized patients receiving peni-

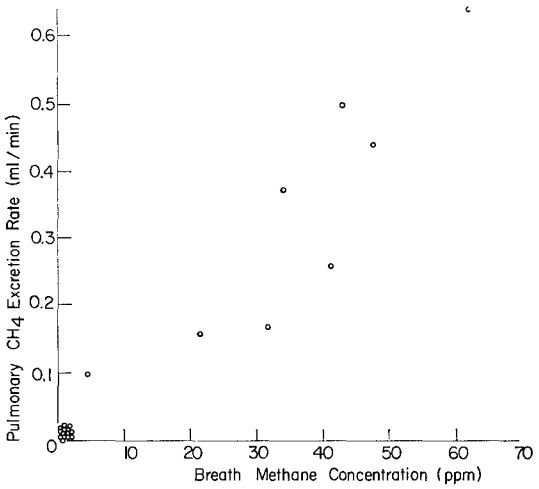

FIG. 3. Correlation between breath CH_4 concentration and the respiratory excretion rate of CH_4 in 22 subjects.

TABLE I

Rate of Methane Formation by Stool Homogenates from Producers and Nonproducers of Methane

Methane producers	Breath [CH_4]	CH_4 from stool incubation
	(*ppm*)	(*ml/hr*)
1	36	1.40
2	54	1.00
3	12	0.41
4	30	0.898
Nonproducers		
1	0.216	0.00016
2	0.156	0.00088
3	0.400	0.000054
4	0.314	0.00020

cillin, tetracycline, or ampicillin; and (*d*) a group of 27 military recruits from a rural area of Minnesota. The percentage of CH_4 producers in each of these groups was 35.3%, 31.7%, 32.5%, and 29.6%, respectively. None of these values differs significantly from the overall mean of 33.6%.

Stability of CH_4-Producing Status.—Breath CH_4 concentrations were studied intermittently over a 1 yr period in 12 subjects. As shown in Table II all sub-

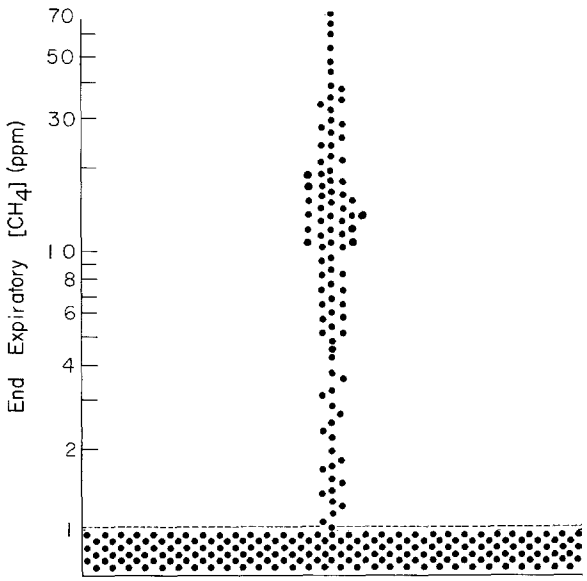

FIG. 4. End-expiratory CH_4 concentrations of 280 adults.

TABLE II

Range of Breath CH_4 Concentrations in 12 Adults Studied Intermittently for a 1 Yr Period

Subject	Breath [CH_4]
	(*ppm*)
1	50–71
2	44–60
3	1.8–4.0
4	38–58
5	4.2–10
6	22–41
7	0.096–0.26
8	0.40–0.66
9	0.16–0.51
10	0.34–0.71
11	0.10–0.43
12	0.21–0.40

jects remained in their producer or nonproducer states, although fluctuations in breath CH_4 concentrations probably not attributable to methodologic errors were noted in these individuals. Over a 4 hr period, the rate of CH_4 excretion appeared to remain extremely constant, as exemplified by the CH_4 excretion rate of the subject shown in Fig. 5.

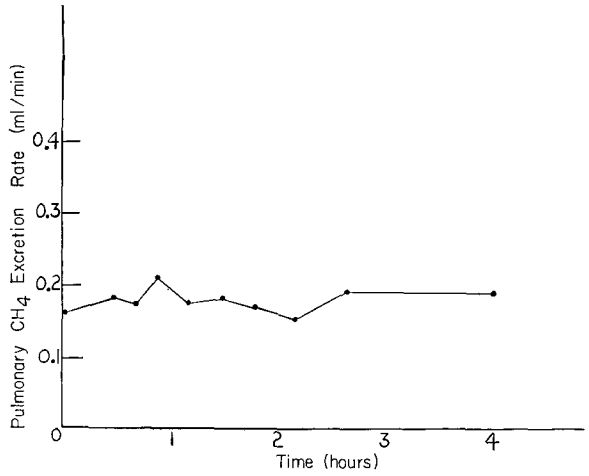

FIG. 5. Pulmonary CH_4 excretion rate of a subject studied over a 4 hr period.

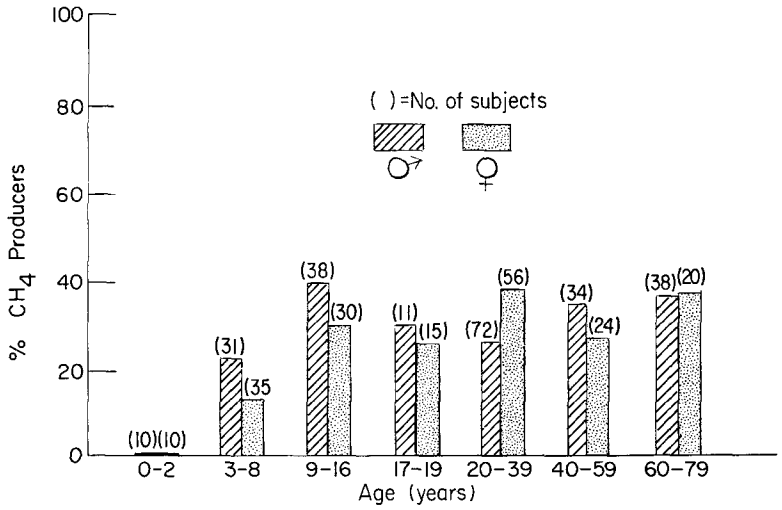

FIG. 6. Influence of age and sex on the incidence of methane production.

Factors Influencing Breath CH_4 Excretion.—

Age and sex: The influence of age and sex on CH_4 production is shown in Fig. 6. Newborns and children below the age of 2 yr excrete no CH_4. The incidence of CH_4 production then increases until at the age of 10 the adult distribution (approximately 33% producers) is reached and subsequently maintained through the 8th decade. Sex (see Fig. 6) had no significant effect on CH_4 production.

Ingestion of nonabsorbable carbohydrates: Previous studies (3) have indicated that H_2 production in the colon depends to large extent upon the quantity of nonabsorbable carbohydrate delivered to the colonic bacteria. Ingestion of lactulose, a nonabsorbable disaccharide, did not influence the breath CH_4 excretion of individuals studied over a 5 hr period (see Fig. 7). In contrast, H_2 excretion was markedly increased when this fermentable substrate was supplied to the colonic bacteria.

Blood group secretor status: The secretor status for A, B, and H substances

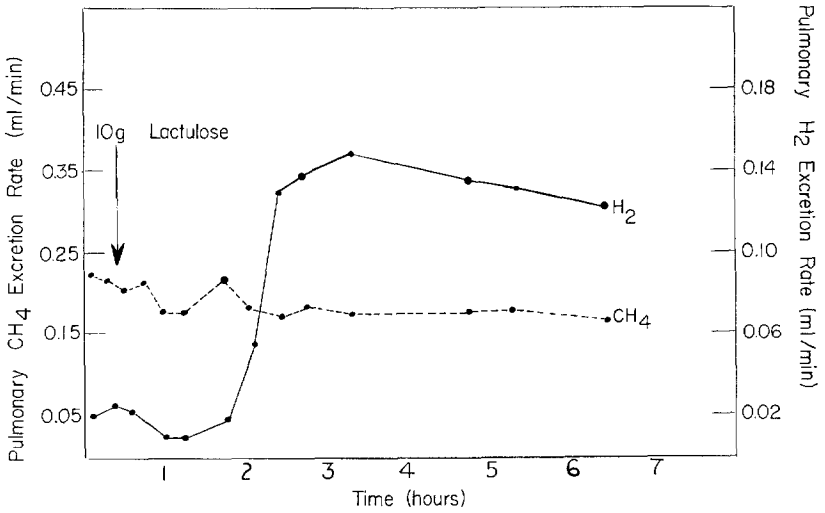

FIG. 7. Effect of the ingestion of lactulose, a nonabsorbable disaccharide, on pulmonary excretion of CH_4 and H_2.

was determined in 10 CH_4 producers; 8 were found to be secretors and 2 were nonsecretors, which is roughly similar to the expected frequency in the general population.

Family Studies and Twins.—There was a strong correlation between an individual's CH_4-producing status and the status of other members of his family. Fig. 8 shows that 84% of 120 siblings of producers of CH_4 also were producers, compared with only 18% of 138 siblings of nonproducers. This difference is highly significant ($P < 0.0001$). 5 of the CH_4 producers had been separated from their family units for from 4 to 30 yr. Breath samples were collected from 13 of their siblings and 92% ($^{12}/_{13}$) of these individuals were also producers of CH_4.

There was also a correlation between CH_4 production by parents and CH_4 production by their offspring. Fig. 8 shows that if both parents were positive

for CH_4 production, $^{24}/_{26}$ or 92% of their children were also positive. When one parent was positive and one negative for CH_4 production, roughly half, or 52% of 34 offspring were positive. When both parents were negative, only 6% of their children were positive. There were seven families where children produced CH_4

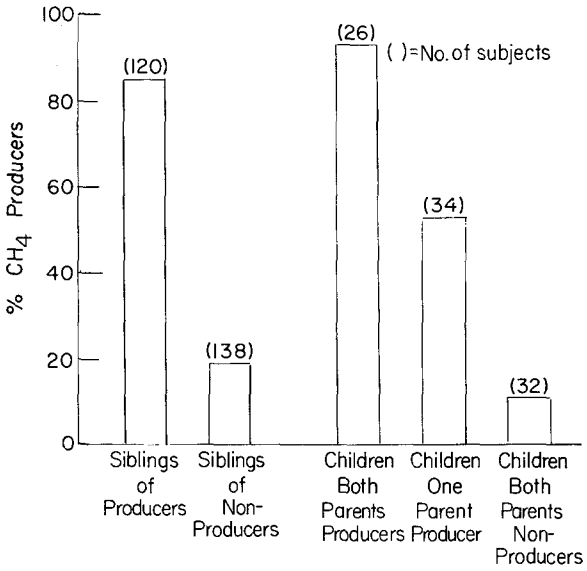

FIG. 8. Incidence of methane production in siblings and children of producers and nonproducers of methane.

TABLE III

Methane Production in Spouses (40 Couples Tested)

CH_4-producing status	Found	Expected*
	%	%
Both producers	18	11
Both nonproducers	42	44
One producer, one nonproducer	40	44

* Expected per cent assuming 33.6% population are CH_4 producers.

and only one of the parents was a producer. In each of the seven families, the mother was the parent who produced CH_4.

Contrasted with this high concordance for CH_4 production between siblings and between parents and their children was the situation observed in spouses (see Table III). The distribution of CH_4 production in these 40 couples was not significantly different ($P > 0.05$) from the distribution that would occur randomly assuming that 33.6% of the population produce CH_4.

In an attempt to better define this familial clustering of CH_4 production, 36 pairs of twins were studied (25 fraternal, 11 identical). 91% of identical and 96% of fraternal twins were concordant for CH_4 production. An important finding was that one pair of apparently identical twins was discordant for methane production. This pair of twins had identical major and four minor blood types.

Institutionalized Subjects.—In order to evaluate the effect of a common environment on CH_4 production, two groups of institutionalized subjects were studied. These individuals, from a veterans' home and a school for the mentally retarded, had lived together in closed units for long periods of time. Fig. 9 shows

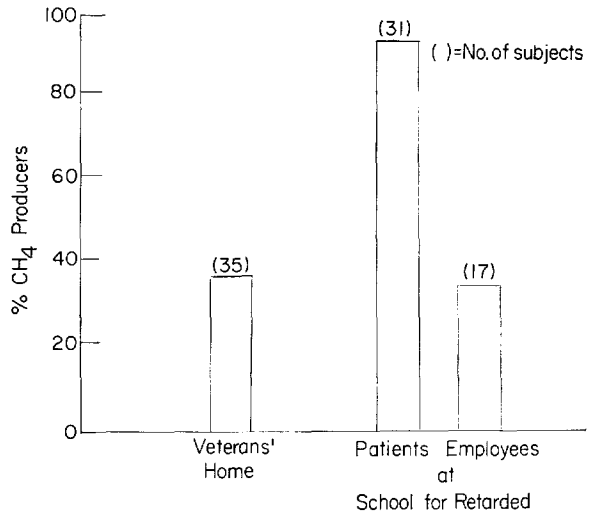

FIG. 9. Incidence of methane production in a veterans' home and a school for the mentally retarded.

the per cent of CH_4 producers in each group. While the expected percentage, roughly 33%, of the individuals from the veterans' home were producers of CH_4, a very high number (93%) of the mentally retarded subjects were positive. A group of employees of this latter institution, who served as controls, had the normal incidence of CH_4 production (35%).

DISCUSSION

The measurement of respiratory CH_4 excretion appears to provide a unique, but simple, technique for the study of the *in situ* metabolism of certain colonic organisms. Compared with the measurement of other bacterial metabolites, CH_4 has several advantageous features. First, the studies carried out in germ-free rats and newborn infants suggest that all CH_4 excreted by man is derived

from bacterial metabolism. Second, we were unable to demonstrate CH_4 utilization by man. Studies in sheep suggested that perhaps 0.33% of intra-arterially infused $^{14}CH_4$ might be converted to $^{14}CO_2$ (6). Such a low rate of CH_4 utilization would not have been detected by the technique used in this study. Third, because of the insolubility of CH_4 in blood relative to air, 90–95% of this gas is cleared in a single passage through the lungs. Thus, the rate of CH_4 absorption from the colon is readily quantitated by breath CH_4 measurements, The total length of time required for collection and analysis of a breath sample for CH_4 is about 6 min. Thus measurement of CH_4 is technically much simpler than measurement of bacterial metabolites that remain in the fecal stream.

The accuracy with which breath CH_4 analysis reflects CH_4 production obviously depends upon whether there is a relatively fixed relationship between CH_4 production and absorption. While this relationship has not been directly investigated, a previous study (3) showed that breath H_2 served as a relatively accurate indicator of colonic H_2 production. H_2, like CH_4, is derived almost entirely from bacterial metabolism in the colon. A mean of 21 ± 9.5% (1 SD) of the H_2 produced in the colon was absorbed and subsequently excreted by the lungs. The rate of colonic absorption of CH_4 should be similar to H_2, although the exact absorption rate cannot be calculated without knowledge of the relative limitation of diffusion versus perfusion in the uptake of gases from the human colon. If blood flow is limiting, the rate of uptake of these gases would be determined by their relative solubilities in blood, and CH_4 would be absorbed about 1.5 times more rapidly than H_2. On the other hand, if diffusion is limiting, the relative diffusivity of these gases in tissue would be rate limiting and H_2 would be absorbed about two times as rapidly as CH_4.[5] H_2 and CH_4 are absorbed at about equivalent rates from the colon of the rat (7).

Thus, it seems likely that respiratory CH_4 can serve as an accurate indicator of CH_4 production in the colon and roughly 20% of the colonic production will appear on the breath. The rapid absorption and subsequent pulmonary excretion of CH_4 means that breath excretion will rapidly reflect changes in CH_4 production. The stability of breath CH_4 excretion observed in our subjects thus reflects a stability of production rate rather than absorption from a large methane pool.

The CH_4-producing bacteria in man have received little study. Most of our knowledge concerning these organisms has been derived from studies of CH_4-producing organisms of ruminants. CH_4 production in these animals may exceed 600 liters/day, which represents an appreciable wastage of calories (8). The highest CH_4 excretor in our group of subjects exhaled about 0.64 ml of CH_4/min or about 900 ml/24 hr. Assuming that exhaled CH_4 represent 20% of the

[5] Diffusivities are calculated from Graham's Law.

total CH_4 excreted, this subject excreted about 4500 ml/day, which represents about 6.0 kcal. Thus, no appreciable loss of calories takes place via CH_4 excretion in man.

All CH_4-producing organisms are strict anaerobes (9). The limitation of CH_4-producing organisms to the colon, and possibly primarily the distal colon, may reflect the requirement of these organisms for a very low oxidation-reduction potential which is probably obtained only in the colon. The only reported attempt to isolate CH_4-producing organisms from man (10) yielded a strictly anaerobic organism with the characteristics of *Methanobacterium ruminatium*, a bacteria which is present in high concentration in the bovine rumen (11).

All CH_4-producing organisms studied to date liberate CH_4 by reduction of CO_2 with H_2 (9). The H_2 can be exogenously added to the culture or formed by bacteria through oxidation of short chain fatty acids or alcohols. The importance of this metabolic pathway in the human colon is uncertain, however, since ingestion of lactulose, which is fermented in the large intestine to H_2 and short chain fatty acids, (12) did not influence the rate of CH_4 excretion. The extreme stability of the rate of CH_4 production is in sharp contrast to H_2 production which fluctuates markedly with meals. Total breath hydrocarbons, which probably represent primarily CH_4, have also been reported to remain relatively constant throughout the day (13).

It seems clear from the present study, as well as a study of total breath hydrocarbons (13), that there are persistent, marked, individual differences in the rate of CH_4 production which persist over long periods of time. There are several possible explanations for these variations in CH_4 production. The most likely possibility would appear to be that a high CH_4 production results from colonization of the colon with large numbers of CH_4-producing bacteria. Preliminary studies by Nottingham and Hungate (10) suggested that a high breath CH_4 excretion might correlate with a high concentration of CH_4-producing organisms.

It is also theoretically possible that all subjects harbor a flora capable of producing CH_4; however, factors such as lack of substrate availability or conditions within the colon (i.e. pH, O_2 tension, or oxidation-reduction potential) inhibit or potentiate the production of this gas. It seems unlikely that such substrate availability is related to dietary differences since spouses and residents of a veterans' home showed no increased concordance for CH_4 production despite the ingestion of relatively similar diets. It is possible that substrates derived from intestinal secretions might account for differences in CH_4 production. An example is the finding of intestinal bacteria capable of metabolizing blood group substances in subjects who secrete these substances into the bowel (14). A brief survey of our subjects showed no obvious relationship between blood group secretor status and CH_4 production.

The possibility that factors such as differences in pH or Po_2 within the colon

could account for differences in CH_4 production appears to be ruled out by the study of fecal homogenates where differences in CH_4 production were noted in the face of a constant pH and, presumably, a similar Po_2.

Whatever the cause of individual differences in CH_4 production, it is apparent that some familial factor has a strong influence on a subject's ability to produce CH_4. While the high concordance for CH_4 production between siblings, as well as parents and offspring, might suggest a genetic influence, the finding of one pair of apparently identical twins discordant for CH_4 production speaks against a totally genetically determined trait. More important, the finding of an extremely high incidence of CH_4 producers in a school for the mentally retarded indicates that under appropriate environmental conditions most subjects can be converted to a CH_4-producing status.

Thus, while we cannot entirely rule out some genetic influence, it appears that environmental factors may be of major importance in determining whether a subject produces CH_4. The lack of concordance for CH_4 production between spouses even though they shared similar environments for many years, as opposed to the high concordance between siblings, suggests that some environmental factor exerts its influence early in life. This early environmental influence appears to permanently establish a subject's CH_4-producing status since subjects separated from their family units for many years maintained the CH_4-producing status of their siblings.

How an early environmental factor might produce this lifelong tendency to CH_4 production is entirely speculative. One possible explanation might be drawn from the work of Mushin and Dubos (15) who demonstrated that the intestinal tract of young mice are readily colonized with a small inoculum of *Escherichia coli* while even large inocula of the same organism failed to infect most adult animals. Furthermore adult mice rarely acquired the bacterial flora of other adult mice with which they came in close contact. Possibly in man also, contact at an early age with CH_4-producing bacteria results in colonization of the colon with these bacteria while adults are resistant to such infection. Once established, these methane-producing bacteria could persist for long periods of time. The high concordance for methane production between parents and children suggests that the parents may act as the source of these bacteria. In support of this concept was the finding that the mother appears to have a greater influence on the CH_4-producing status of the children than does the father.

The high percentage of methane producers from the institutionalized population may result from close contact and poor personal hygiene. These conditions may allow repeated inoculation with unusually large numbers of methane-producing bacteria leading to eventual colonization.

Several investigators have reported the striking stability of the fecal flora of individual subjects, even when these subjects ingested identical diets (16).

Lerner et al. detected no significant change in the microflora of 10 adults studied serially over 5 months (17). Others have confirmed the persistence over many weeks of a given serotype of *E. coli* in both infants (18) and adults (19). Furthermore, attempts to introduce a foreign serotype was usually unsuccessful. Our studies using breath methane excretion as an indicator of the presence of methane-producing bacteria provide indirect evidence that the number of these bacteria may remain relatively stable over long periods of time. Indeed, these organisms may become established early in life and then persist through adulthood. Long-range, prospective studies would be necessary to prove this conclusion, however.

It is commonly assumed that CH_4 is inert and exerts its toxic effect solely on the basis of asphyxia. However, the study of Dougherty, O'Toole, and Allison (6) suggests that small quantities of CH_4 may enter metabolic pathways, and there also is evidence that CH_4 may interfere with certain types of hydrogen bonding, such as occurs in sickling erythrocytes (20). While the CH_4 tension (P_{CH_4}) of portal blood was not measured, this tension can be readily calculated from knowledge of the solubility of CH_4 in blood (2.4 ml/100 ml per 760 mm Hg)[6] and the rate of CH_4 excretion on the breath. Assuming a portal blood flow of 1000 ml/min, a subject who expires 0.4 ml of CH_4/min will have a P_{CH_4} in portal blood of about 12 mm Hg. Thus, the liver of the CH_4 producer is chronically exposed to a sizeable CH_4 tension via what might be termed internal pollution. The P_{CH_4} in the colon, as indicated by studies of the composition of flatus, may reach levels of 200 mm Hg (21). The possible detrimental or beneficial effects of such chronic methane exposure have not been investigated.

Independent of the possible toxicity of CH_4, the present studies have certain implications regarding the influence of intestinal bacterial metabolism in health and disease. It is apparent from measurements of breath CH_4 excretion that differences in bacterial metabolism can result in relatively enormous differences in the exposure of the host to bacterial metabolites, both locally within the colon and systemically via absorption of the metabolites. These differences in exposure may be chronic and determined by familial factors. As has been proposed by Dubos (1), such differences in intestinal bacterial metabolism could readily have a profound, but rarely recognized, influence on the host.

SUMMARY

Measurements of pulmonary excretion of methane (CH_4) were used to obtain information on the CH_4-producing bacteria in man. Preliminary studies indicated that (*a*) all CH_4 excreted by man is produced by colonic bacteria, (*b*) there is no appreciable utilization of CH_4 by man, and (*c*) breath CH_4 can serve as a relatively accurate indicator of CH_4 production in the intestine.

[6] This figure represents solubility of CH_4 in H_2O at 37°C which should not differ appreciably from its solubility in blood.

The rate of pulmonary CH_4 excretion varied enormously, ranging from undetectable ($< 5 \times 10^{-6}$ ml/min) to 0.66 ml/minute. In general, the CH_4 excretion rate for subjects was consistently very low (nonproducers) or relatively large (producers). 33.6% of the adult population were producers of CH_4. Whereas diet, age over 10 yr, and sex did not influence the rate of CH_4 production, some familial factor appeared to play an important role. 84% of siblings of CH_4 producers also were producers, while only 18% of the siblings of nonproducers were found to be CH_4 producers. This familial tendency appeared to be determined by early environmental rather than genetic factors.

These studies of CH_4 excretion demonstrate that the exposure of individuals to intestinal bacterial metabolites may differ markedly and that these differences may be chronic and determined by familial factors.

We thank the Minneapolis Mothers of Twins Club for their help in facilitating these investigations.

BIBLIOGRAPHY

1. Dubos, R., R. W. Schaedler, R. Costello, and P. Hoet. 1965. Indigenous, normal, and autochthonous flora of the gastrointestinal tract. *J. Exp. Med.* **122:**67.
2. Coburn, R. F., W. J. Williams, and S. B. Kahn. 1966. Endogenous carbon monoxide production in patients with hemolytic anemia. *J. Clin. Invest.* **45:**460.
3. Levitt, M. D. 1969. Production and excretion of hydrogen gas in man. *New Engl. J. Med.* **281:**122.
4. Fordtran, J. S., R. Levitan, V. Bikerman, B. A. Burrows, and F. J. Ingelfinger. 1961. The kinetics of water absorption in the human intestine. *Trans. Ass. Amer. Physicians* **74:**195.
5. Engel, R. R., and M. D. Levitt. 1970. Intestinal trace gas formation in newborns. Am. Ped. Soc. and Soc. for Ped. Res. Combined Program and Abstracts. 266 (Abstr.)
6. Dougherty, R. W., J. J. O'Toole, and M. J. Allison. 1967. Oxidation of intra-arterially administered carbon^{14}-labelled methane in sheep. *Proc. Soc. Exp. Biol. Med.* **124:**1155.
7. Levitt, M. D. 1969. Use of gases for study of the intestinal circulation. *Clin. Res.* **17:**306. (Abstr.)
8. Hungate, R. E., D. W. Fletcher, and R. W. Dougherty. 1955. Microbial activity in the bovine rumen: its measurement and relation to bloat. *Appl. Microbiol.* **3:**161.
9. Stadtman, T. C. 1967. Methane fermentation. *Annu. Rev. Microbiol.* **21:**121.
10. Nottingham, P. M., and R. E. Hungate. 1968. Isolation of methanogenic bacteria from feces of man. *J. Bacteriol.* **96:**2178.
11. Smith, P. H., and R. E. Hungate. 1958. Isolation and characterization of Methanobacterium ruminatium. *J. Bacteriol.* **75:**713.
12. Bircher, J., J. Muller, P. Guggenheim, and U. P. Haemmerli. 1966. Treatment of chronic portal-systemic encephalopathy with lactulose. *Lancet.* **1:**890.
13. Calloway, D. H., and E. L. Murphy. 1968. The use of expired air to measure intestinal gas formation. *Ann. N.Y. Acad. Sci.* **150:**82.

14. Hoskins, L. C. 1969. Ecologic studies of intestinal bacteria. Relations between the specificity of fecal ABO blood group antigen-degrading enzymes from enteric bacteria and the ABO blood groups of the human host. *J. Clin. Invest.* **48**:664.
15. Mushin, R., and R. Dubos. 1965. Colonization of the mouse intestine with escherichia coli. *J. Exp. Med.* **122**:745.
16. Zubrzycki, L., and E. H. Spaulding. 1962. Studies on the stability of the normal human fecal flora. *J. Bacteriol.* **83**:968.
17. Lerner, P., S. Gorbach, L. Nahas, and L. Weinstein. 1966. Stability of the normal human intestinal micro-flora. *Clin. Res.* **14**:301. (Abstr.)
18. Gage, R., C. B. Gunther, and E. H. Spaulding. 1961. Persistence of E. Coli in the stools of young infants. *Bacteriol. Proc.* 117. (Abstr.)
19. Sears, H. J., I. Brownlee, and J. K. Uchiyama. 1950. Persistence of individual strains of escherichia coli in the intestinal tract of man. *J. Bacteriol.* **59**:293.
20. Murayama, M. 1964. A molecular mechanism of sickled erythrocyte formation. *Nature (London).* **202**:258.
21. Kirk, E. 1949. Quantity and composition of human colonic flatus. *Gastroenterology.* **12**:782.