FACTORS INFLUENCING PULMONARY METHANE
EXCRETION IN MAN

AN INDIRECT METHOD OF STUDYING THE IN SITU METABOLISM
OF THE METHANE-PRODUCING COLONIC BACTERIA*

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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 metabo-
lism 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 metabo-
lite, methane (CH₄), 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 CH₄
excreted by man is derived from the metabolism of intestinal bacteria, and (b)
there is no appreciable utilization of CH₄ by man; thus, all CH₄ absorbed from
the colon will be quantitatively eliminated by the lungs.

In this investigation we first attempted to demonstrate that pulmonary CH₄
excretion can serve as an accurate indicator of CH₄ 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 CH₄ was studied in
20 infants ranging in age from 2 hr to 5 months and in 22 normal adults. The CH₄ concen-
tration 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.

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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 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 rebreathe 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 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 Tedlolyne oxygen analyzer, adjusted to maintain a Po$_2$ of 150 ± 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 (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$_2$O-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 hyperventilation. The gas samples were analyzed for O$_2$ as well as CH$_4$ and only those collections with a Po$_2$ 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
has previously been described (3) for the study of intestinal H₂ 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 hexafluoride (SF₆), was constantly infused into the bowel via the proximal orifice of the tube at a rate of 30 ml/min. SF₆ 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 CH₄ 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 CH₄ 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 CH₄. 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 CH₄ excretion in adults was determined using argon as a carrier gas, and thermal conductivity and hydrogen-flame detectors were connected in series to measure O₂ and CH₄ respectively. Measurement of CH₄, H₂, and SF₆ in the intestinal perfusion and the rat studies employed a thermal conductivity detector.

RESULTS

Site and Rate of CH₄ Production in the Bowel.—During the intestinal perfusion experiments CH₄ was never detected in gas sampled from the mid-jejunum or ileum. In 5 of the 14 subjects, CH₄ was readily detected in gas passed via rectum with the fasting rate of colonic CH₄ production of these 5 subjects averaging 0.45 ± 0.13 (1 SE) ml/min. In the other 9 subjects CH₄ was not detectable (<100 ppm) indicating a CH₄ production of less than 0.003 ml/min. The rate of CH₄ production was not significantly altered by the infusion of lactose into the bowel.

In one CH₄-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 H₂ production indicated that about 57% of the colonic H₂ production occurred proximal to this collecting site.

Bacterial Origin of CH₄ Production.—In an attempt to prove that CH₄ is solely derived from bacterial metabolism, studies were carried out in germfree rats and newborn infants. As shown in Fig. 1, CH₄ excretion was not detected
by rats in the germfree state; however, CH₄ excretion became detectable within 5 days of contamination of these rats with fecal material from a rat previously shown to produce large quantities of CH₄. CH₄ excretion was never detected (<6 × 10⁻⁶ ml/min) in infants up to 6 months of age. In contrast, another bacterial product (H₂) was detected within 24 hr of life (5).

Utilization of CH₄.—In order to determine if CH₄ was metabolized by man, the rate of removal from the closed system of exogenously added CH₄ was studied in three nonproducers. In each of these subjects, the concentration of CH₄ and He fell at identical rates indicating no detectable utilization of CH₄.

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**FIG. 1.** Methane excretion of three rats in the germfree state and after contamination with fecal material from a CH₄-producing rat.

**Pulmonary Excretion Rate of CH₄.**—The rate of pulmonary CH₄ 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 CH₄ excretion, ranging from undetectable (<5 × 10⁻⁶ ml/min) up to 0.66 ml/min. In general, subjects excreted little or no CH₄, or relatively large quantities of CH₄. This finding suggested that measurement of the concentration of CH₄ in expired air might serve as a sufficiently accurate indicator of CH₄ excretion for epidemiologic studies.

**Measurement of CH₄ Concentration in End-Expiratory Air.**—A variety of techniques were tested in an attempt to obtain the most reproducible and accurate measurement of alveolar CH₄ concentration. Because of the low solubility of CH₄ in blood relative to air, transient hyperventilation before the collection of breath samples would rapidly wash out the CH₄ 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 ±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.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{Pulmonary CH$_4$ excretion rate of 22 adults.}
\end{figure}

_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 penicillin, tetracycline, or ampicillin; and (d) a group of 27 military recruits from a rural area of Minnesota. The percentage of CH₄ 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 CH₄-Producing Status.—Breath CH₄ concentrations were studied intermittently over a 1 yr period in 12 subjects. As shown in Table II all sub-

![Graph](image-url)

**Fig. 3.** Correlation between breath CH₄ concentration and the respiratory excretion rate of CH₄ in 22 subjects.

**TABLE I**

| Methane producers | Breath [CH₄] (ppm) | CH₄ from stool incubation (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                          |
TABLE II

Range of Breath CH₄ Concentrations in 12 Adults Studied Intermittently for a 1 Yr Period

| Subject | Breath [CH₄] (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       | 0.16-0.31         |
| 10      | 0.34-0.71         |
| 11      | 0.10-0.43         |
| 12      | 0.21-0.40         |

Subjects remained in their producer or nonproducer states, although fluctuations in breath CH₄ concentrations probably not attributable to methodologic errors were noted in these individuals. Over a 4 hr period, the rate of CH₄ excretion appeared to remain extremely constant, as exemplified by the CH₄ excretion rate of the subject shown in Fig. 5.
Fig. 5. Pulmonary CH₄ 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 CH₄ Excretion.—

Age and sex: The influence of age and sex on CH₄ production is shown in Fig. 6. Newborns and children below the age of 2 yr excrete no CH₄. The incidence of CH₄ 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 CH₄ production.
Ingestion of nonabsorbable carbohydrates: Previous studies (3) have indicated that H\textsubscript{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\textsubscript{4} excretion of individuals studied over a 5 hr period (see Fig. 7). In contrast, H\textsubscript{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 was determined in 10 CH\textsubscript{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\textsubscript{4}-producing status and the status of other members of his family. Fig. 8 shows that 84% of 120 siblings of producers of CH\textsubscript{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\textsubscript{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\textsubscript{4}.

There was also a correlation between CH\textsubscript{4} production by parents and CH\textsubscript{4} production by their offspring. Fig. 8 shows that if both parents were positive
for CH₄ production, 24% or 92% of their children were also positive. When one parent was positive and one negative for CH₄ 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 CH₄, and only one of the parents was a producer. In each of the seven families, the mother was the parent who produced CH₄.

Contrasted with this high concordance for CH₄ production between siblings and between parents and their children was the situation observed in spouses (see Table III). The distribution of CH₄ 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 CH₄.

![Graph showing incidence of methane production in siblings and children of producers and non-producers of methane.](image)

**Fig. 8.** Incidence of methane production in siblings and children of producers and non-producers of methane.

**TABLE III**

*Methane Production in Spouses (40 Couples Tested)*

| CH₄-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 CH₄ producers.
In an attempt to better define this familial clustering of CH₄ production, 36 pairs of twins were studied (25 fraternal, 11 identical). 91% of identical and 96% of fraternal twins were concordant for CH₄ 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 CH₄ 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

![Graph showing the percentage of CH₄ producers in veterans' home and school for retarded individuals.](image)

The per cent of CH₄ producers in each group. While the expected percentage, roughly 33%, of the individuals from the veterans' home were producers of CH₄, 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 CH₄ production (35%).

**DISCUSSION**

The measurement of respiratory CH₄ 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, CH₄ has several advantageous features. First, the studies carried out in germ-free rats and newborn infants suggest that all CH₄ excreted by man is derived
from bacterial metabolism. Second, we were unable to demonstrate CH₄ utilization by man. Studies in sheep suggested that perhaps 0.33% of intravenously injected ¹⁴CH₄ might be converted to ¹⁴CO₂ (6). Such a low rate of CH₄ utilization would not have been detected by the technique used in this study. Third, because of the insolubility of CH₄ in blood relative to air, 90-95% of this gas is cleared in a single passage through the lungs. Thus, the rate of CH₄ absorption from the colon is readily quantitated by breath CH₄ measurements. The total length of time required for collection and analysis of a breath sample for CH₄ is about 6 min. Thus measurement of CH₄ is technically much simpler than measurement of bacterial metabolites that remain in the fecal stream.

The accuracy with which breath CH₄ analysis reflects CH₄ production obviously depends upon whether there is a relatively fixed relationship between CH₄ production and absorption. While this relationship has not been directly investigated, a previous study (3) showed that breath H₂ served as a relatively accurate indicator of colonic H₂ production. H₂, like CH₄, is derived almost entirely from bacterial metabolism in the colon. A mean of 21 ± 9.5% (1 sd) of the H₂ produced in the colon was absorbed and subsequently excreted by the lungs. The rate of colonic absorption of CH₄ should be similar to H₂, 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 CH₄ would be absorbed about 1.5 times more rapidly than H₂. On the other hand, if diffusion is limiting, the relative diffusivity of these gases in tissue would be rate limiting and H₂ would be absorbed about two times as rapidly as CH₄. H₂ and CH₄ are absorbed at about equivalent rates from the colon of the rat (7).

Thus, it seems likely that respiratory CH₄ can serve as an accurate indicator of CH₄ production in the colon and roughly 20% of the colonic production will appear on the breath. The rapid absorption and subsequent pulmonary excretion of CH₄ means that breath excretion will rapidly reflect changes in CH₄ production. The stability of breath CH₄ excretion observed in our subjects thus reflects a stability of production rate rather than absorption from a large methane pool.

The CH₄-producing bacteria in man have received little study. Most of our knowledge concerning these organisms has been derived from studies of CH₄-producing organisms of ruminants. CH₄ production in these animals may exceed 600 liters/day, which represents an appreciable wastage of calories (8). The highest CH₄ excretor in our group of subjects exhaled about 0.64 ml of CH₄/min or about 900 ml/24 hr. Assuming that exhaled CH₄ represent 20% of the

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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 ruminantium*, 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.

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$^6$ This figure represents solubility of CH$_4$ in H$_2$O at 37°C which should not differ appreciably from its solubility in blood.
The rate of pulmonary CH₄ excretion varied enormously, ranging from undetectable (<5 × 10⁻⁶ ml/min) to 0.66 ml/minute. In general, the CH₄ excretion rate for subjects was consistently very low (nonproducers) or relatively large (producers). 33.6% of the adult population were producers of CH₄. Whereas diet, age over 10 yr, and sex did not influence the rate of CH₄ production, some familial factor appeared to play an important role. 84% of siblings of CH₄ producers also were producers, while only 18% of the siblings of nonproducers were found to be CH₄ producers. This familial tendency appeared to be determined by early environmental rather than genetic factors.

These studies of CH₄ 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.

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