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The biological significance of gaseous biomarkers from the microbiota in the alimentary tract.
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WHAT DO WE KNOW FROM GERMFREE LIFE? BASIC KNOWLEDGE ABOUT MICROBES AND GAS PRODUCTION

TORE MIDTVEDT

Department of Microbiology, Tumour and Cell Biology,
Karolinska Institute, Stockholm, Sweden

SUMMARY

The gases within the alimentary lumen reflect the composition and volume of swallowed respiratory air or gases, the release of gases from food, the kinds and amounts of non-respiratory gases produced within the alimentary tract by its microbiota, and the rate of gaseous exchange between the alimentary lumen and surrounding blood vessels and tissue. Basic information about microbial produced gases was obtained some decades ago. The physiological and pathophysiological influences that these gases might have on the host will be outlined in detail in the following chapters. On-going and future technological improvements will give us valuable tools for studying these continuously ongoing host-microflora cross-talks.

INTRODUCTION

All multi-cellular organisms with an alimentary tract have gas in their tract. Principally, its origin may be from the following sources:

1. Swallowed as air,
2. Derived from gaseous compounds in the diet,
3. Diffusion into the tract from blood vessels and surrounding tissue, and
4. Being produced by the alimentary microbiota.

During this seminar other speakers focussed upon microbial production of gases. In order to put this production into its physiological and pathophysiological frame, some general comments and introduction of some terms will be given. From a historical point of view, the basic differences between conventional and germfree animals with regard to alimentary gas production was worked out in the 1960-ties due to one simple reason: Man was entering outer space and recognized that this caused some abdominal discomfort. Great efforts were made to overcome this discomfort and germfree animal laboratories in several countries became involved. The present list of references and Figure 1 reflect to some extend the interest and efforts of 40 years ago.

COMPOSITION OF ALIMENTARY GAS

Comparative studies in germfree and conventional animals have clearly shown that the microbiota alone are responsible for presence of hydrogen,

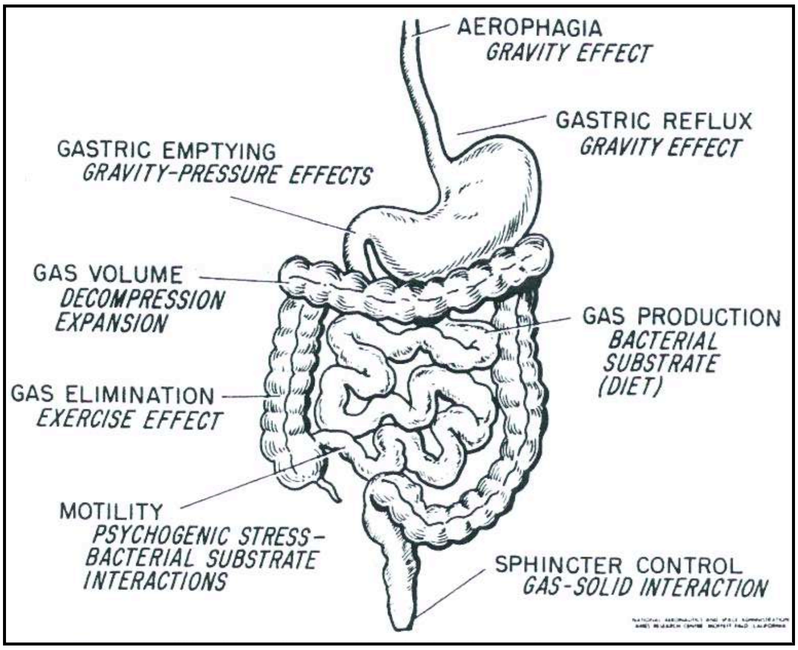

Figure 1: Alimentary tract problems that man encounters in space.

methane, hydrogen sulphide, some of the volatile fatty acids and amines as indole, skatole etc. The major differences between the microbial produced gases and the others that might be present in the GI tract is that the former reach their highest concentration within the lumen and will always diffuse out from the lumen, most often to be removed to the atmosphere from the transporting blood as it flows through the lung. Consequently, measurements of any of these gases in respired air reflect an alimentary production and this can be of diagnostic importance. The absolute and relative amounts of the microbial produced gases vary profoundly with the types and abundance of microorganisms present in the various compartments of the alimentary tract and with the substrate provided for their growth.

The other gases, regardless whether they are derived *per os* or via the blood stream may diffuse both into and out of the lumen and their presence in respired air does not reflect a microbial alimentary origin.

The net sum of all these processes yields a rest of gases to be expelled as flatus. A simple rule of the thumb says that around 2/3 of the gases in flatus originates from the host whereas 1/3 is of microbial origin. In the following, attempts will be made to follow the gas on its way through the alimentary tract.

UPPER ALIMENTARY TRACT

Air swallowing, aerophagia, is a universal phenomenon in all mammals. In man, the relative amount varies considerably. However, there is considerable evidence that swallowed air accounts for more than half of the gastro-

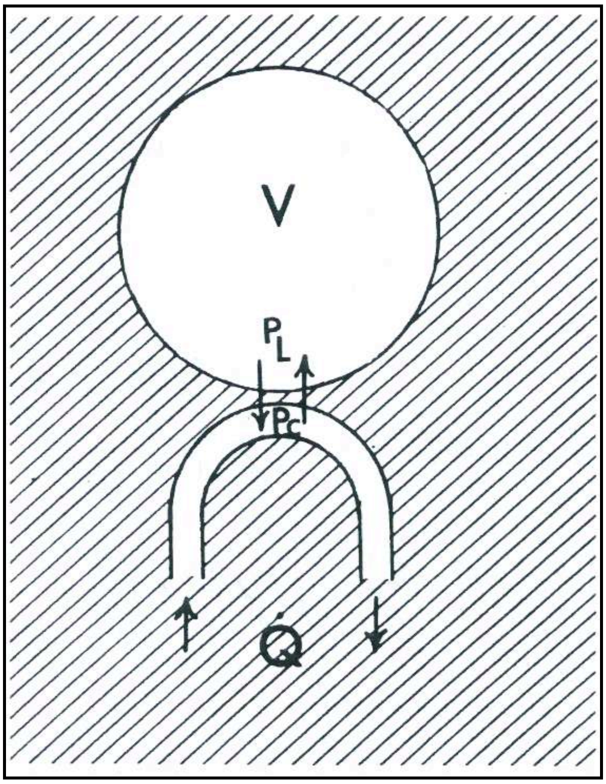

Figure 2: Exchange of gas between lumen and tissue.
Q = tissue, V = volume of gas in the gut. P_L = partial pressure of a gas in the lumen; P_C =Partial pressure of same gas in venous blood/surrounding tissue.

intestinal "gas" in man. Increased aerophagia is sometimes referred to as *eructatio nervosa*. This designation implies that frequent eructation may occur on a nervous or psychogenic basis. Whatever the amount of swallowed air might be, it can either be eructated from the oesophagus/stomach, absorbed by the host, utilized by his alimentary microbiota or expelled per rectum as flatus. In the past in our Western culture (it still is in some other cultures) eructation was looked upon as an audible expression of appreciation of the host's culinary accomplishment. Anyhow, eructation may account for a lesser part of swallowed air. Within its way down the tract, the swallowed air equilibrates or approaches equilibrium with in the gases dissolved in blood perfusing the intestines or locally produced or utilized by the microbiota. In any compartment it will attain a volume determined by the relative rates of gas inflow, formation, exchange and expulsion.

Passage of swallowed air and other gases within the alimentary tract is faster than passage of content. Already for more than 75 years ago, it was demonstrated that swallowed air can move from the stomach to the caecum in 6 to 15 minutes and to the rectum in 36 minutes (*Magnusson*, 1931). These times are too short for a complete exchange between some alimentary gases and blood/tissue to take place.

VISIBLE GAS IN THE INTESTINE OF GERMFREE ANIMALS

It is an everyday experience for people working with germfree animals that gas can be seen in both the small and large intestine, especially in the caecum. Depending of where in the alimentary tract the gas bubble is located, the relative content of gases might vary, ending up as mostly N_2 in flatus. The mathematical background for this can be worked out using rather complicated mathematical equations (*Forster*, 1968), but basically can be depicted as done in Figure 2.

Any time - and at any place - the content in a gas bubble will be regulated by influx/efflux, new production and utilization. The relative rate of influx/efflux for the gases of non-microbial origin will always be related to corresponding values in surrounding blood vessels/tissue. Some basic efflux data are given in Table 1. The data are taken from different publications and based upon studies in various animals species and alimentary compartments but is nevertheless of some value.

In the germfree animal there is no microbial production or utilization of gases. Consequently, the relative difference between N_2, O_2 and CO_2 are of importance. As is evident from the table, N_2 will diffuse far more slowly than O_2 and CO_2, yielding more and more N_2 in the gas bubble on its rapid passage to the anus. Further, the high values of CO_2 might be due to other factors than diffusion, and might vary from species to species (*Rasmussen* et al., 2002). At least in rats, the amounts of carbonic anhydrase enzymes do not seem to be influenced upon by the microbiota (*Lonnerholm* et al., 1988)

The data in Table 1 might also be used when explaining the old clinical experience that it may help postoperatively to give the patients some oxygen. Inhalation of air with an increased amount of oxygen will denitrogenate blood and tissue, thereby widening the gradient for N_2 across the intestinal mucosal barrier. As a result, intraluminal nitrogen enters the blood stream more rapidly and leaves the body via the lungs, and the intestinal gas volume will decrease concomitantly (*Pogrund* and *Steggerda*, 1948).

Another way to obtain the same result might be to increase the environmental pressure thereby reducing the intraluminal gas volume proportionally and obtaining a rapid relief in discomfort. This works out efficiently in experimental models (*Cross* et al., 1953; *Cross*, 1965) and has been tried on patients with some limited results (*Stewart* et al., 1964). However, a hyperbaric chamber, suitable for treatment of seriously ill patients, is usually not close enough when it is urgently needed.

Following an establishment of small intestinal strangulation obstruction, germfree rats will be alive somewhat longer than their conventional counterparts (*Midtvedt*, 1984). One reason for this difference might be a relatively larger deficiency in oxygen supply to the enterocytes in conventional animals. Intraluminal administration of pure oxygen at one atmosphere of absolute pressure protects three-inch length of ischemic small intestine in conventional rats from gross and histological damage for up to 6 hours (*Gottfried* et al, 1963). By contrast, frank necrosis appeared within some few minutes in control animals. However, these promising results could not be repeated in dogs (*van Zyl*, 1966).

Previously it has also been reported that alteration in environmental pressure and composition of inhaled gas, may influence upon intestinal motility.

Table 1: Relative rates of diffusion from the lumen

Nitrogen	1
Methane	4
Hydrogen	8
Oxygen	11 - 14
Hydrogen sulphide	69
Carbon dioxide	160

These data are derived from experiments on isolated intestinal segments of different animal species, mostly referred to by *Saltsman* and *Sicker*, 1968.

DIETARY INFLUENCE UPON COMPOSITION OF INTESTINAL GAS

"It must be something that I have eaten" has been – and still is – a general explanation for many gastrointestinal disturbances. Out of the many subjective hypotheses, probably the most accurate, is the relationship that exists between the ingestion of some vegetables, as beans, peas, etc, and the production of intestinal gases. This relationship is based upon an influence of the alimentary microbiota upon dietary compounds with a subsequent production of large amounts of various gases. Basis information of this relationship was worked out when man went into space in the 1960-ties, and a simple construction for quantitative and qualitative analysis of flatus was established. Shortly, a catheter was inserted into the rectum some cm beyond the anal sphincter. The end inserted into the rectum was perforated with a number of holes approximately one cm apart and the other end was attached to a cylinder containing an acidified sulphate solution. The volume of flatus passed was recorded by measuring the displacement of fluid in the cylinder. At the end of the collection period, the composition of the gas was analysed. The collection period could vary and so could also their relationship to meals. Data from one out of several experiments are as follows. On a basal diet, the volume of flatus collected was 15 cm^3/h, and percentage of methane was 7.3, i.e. around 1 cm^3/h. After a meal consisting of pork, beans or peas, the total volume increased more than tenfold and the percentage of methane more than doubled i.e. 30 - 40 cm^3/h of methane was produced hourly. It is well known that ruminants produced considerable amounts of methane (hundreds of cm^3 hourly after a meal), which is a gas around 30 times more "toxic" for global warming than CO_2. Consequently, presence of a number of "holy cows "in some countries have been questioned. Probably, methane production by man should also be taken into some consideration. In Sweden, pea soup is traditionally served every Thursday in nearly all restaurants. It goes back some hundred years, since one of their kings (Erik XIV) was killed by eating poisoned pea soup, and the Swedes want to demonstrate that they were innocent. Assuming that more than a million Swedes have pea soup meal every Thursday, the production of human produced methane in Sweden will be some thousands cubic meter of methane, outnumbering many holy cows.

A LIFE WITHOUT ANAEROBIC ALIMENTARY MICROBIOTA

"Life is not possible without bacteria!" That statement, expressed by L Pasteur more than a century ago, is contradicted by the life of germfree mammals. However, a life together with microbiota, but without the anaerobes and only with aerobic metabolism, should be something very special. Firstly, the ruminants would have difficulties to exist, since their yield of energy is based solely on an anaerobic breakdown of cellulose and an anabolic building-up of energy-rich organic acids, to be utilized by the host. In an omnivore as man, very large amounts of air, i.e. oxygen would be needed to serve the aerobic microbiota. In short, we would need to have a respiration as a 100-meter runner just finished. The major endproduct of carbohydrate metabolism would be CO_2 and water. There would be little, if any, need for alterative ways of getting rid of excess of H^+ or electrons, i.e. no methane, H_2 or hydrogen sulphide would be formed, and the need for a recirculation of nitrogen would be diminished and more products would have to be excreted in the urine. In short: More intake of food and air, much more production of urine and flatus. Thus, 1-2 kg of anaerobes has a dramatic influence upon our living.

FUTURE RESEARCH ON GASEOUS BIOMARKERS DERIVED FROM THE MICROBIOTA IN THE ALIMENTARY TRACT

As outlined above, studies on the amounts and composition of alimentary phases may be somewhat troublesome and not always easy to perform. For many years, röntgenology has been a suitable method in clinical medicine to study presence of gas in the alimentary tract (*Felson*, 1968). However, there might be good reasons to believe that even more specific methods are on their way. Future developments in nuclear magnetic resonance (NMR) spectroscopy will allow us to follow gases specific produced by the microbiota on their way from the alimentary tract to their possible places for influences on the host – wherever it might be (intestinal wall, blood vessels, nerve cells etc.). A better understanding of the many gaseous cross talks between a host and his intestinal microbiota represent future challenges and germfree - specifically contaminated ex-germfree - and conventional animals are valuable tools for overcoming these challenges.

Another challenge for the future is microbial utilization of N_2 in swallowed air. Probiotics with that property might reduce the increasing amounts of N_2 all the way down in the alimentary tract, and may reduce the symptoms in some patients with irritable able bowel syndrome.

Assuming that lack of nitrogen reduces a microbial breakdown of cellulose and other carbon-rich molecules, genetically modified mircroorganisms with N_2-fixating properties present in the rumen, would solve the problem. Similarly, probiotics with that property would reduce the demand for proteins in developing countries.

CONCLUSION

Presence of gas in the alimentary tract is a challenge, in physiology as well as pathophysiology. A better understanding of the many microbe/microbe and microbe/host cross-talks that are governed by gaseous molecules throughout the alimentary tract will create possibilities for interventions. Alimentary gases are far more than just flatus.

LITERATURE

Cross, F.A. and Wangensteen, O.H.: The effect of increased atmospheric pressure and the inhalation of 95 per cent oxygen and helium-oxygen mixtures on the viability of the bowel wall and the absorption of gas in closed-loop obstruction. Surg. Forum 5, 111-116 (1953).

Cross, F.A.: Hyperbaric treatment of experimental intestinal obstruction. Dis. Chest 47, 374-381 (1965).

Felson, B.: Abdominal gas: A roentgen approach. Ann. New York Acad. Sci. 150, 141-161 (1968).

Forster, R.E.: Physiological basis of gas exchange in the gut. Ann. New York Acad. Sci. 150, 4-12 (1968).

Gottfried, B., Molomut, N., and Patti, J.: Effect of enterically supplied oxygen on a devascularized loop of ileum. Surgery 53, 484-489 (1963).

Lonnerholm, G., Midtvedt, T., Schenholm, W., and Wistrand, P.J.: Carbonic anhydrase isoenzymes in the cecum and colon of normal and germfree rats. Acta Phys. Scand. 132, 159-166 (1988).

Magnusson, W.: On meteorism in pyeolography and on passage of gas through the small intestine. Acta Radiol. 12, 552-556 (1931).

Midtvedt, T.: A possible role of intestinal mucin in the pathophysiology of intestinal strangulation obstruction. Consequences of tracing a clinical observation. Eur. Surg. Res. 16 (Suppl. 2), 68-72 (1984).

Pfeiffer, C.J.: Gastroenterologic aspects of manned spaceflight: Comments on gastrointestinal gas and environmental stress. Ann. New York Acad. Sci. 150, 40-48 (1968).

Rasmussen, H., Mirtaheri, P., Dirven, H., Johnsen, H., Kvarstein, G., Toennessen, T.I., and Midtvedt, T.: PCO_2 in the large intestine of mice, rats, guinea pigs, and dogs and effects of the dietary substrate. J. Appl. Physiol. 92, 219-224 (2002).

van Zyl, J.J.W.: Hyperbaric oxygenation with special consideration of the surgical applications. Doctoral Thesis. University of Stellenbosch, South Africa. Cited by Saltzmann, H.A. and Sieker, H.O.: Intestinal response to changing gaseous environments: Normobaric and hyperbaric observations. Ann. New York Acad. Sci. 150, 31-39 (1968).