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DEFENCE MECHANISMS OF THE INNATE SYSTEM: NO AS A GASTROINTESTINAL ECO-REGULATOR

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SUMMARY

Nitrogen (N) constitutes around 80% of the air. The air gas di-nitrogen is transformed by soil microorganisms into organic material which is utilised by all other organisms. Denitrification is an important microbiological process for the return of fixed nitrogen to the atmosphere. Non-microbial life exists in between these two major microbial processes.

In most organisms, nitrogen is a limited source and consequently, they have developed systems for excretion and recycling. In ureotelic organisms, as mammals, urea is the major excretion product; uric acid is dominating in birds, and ammonium in organisms as amphibian. In most mammals, parts of urea will be excreted into the gastrointestinal tract where it is used for protein synthesis by the microbial flora. The proteins thus formed might be utilised by the host. Nitrate constitutes an important dietary form of nitrogen.

Despite having no host enzymes capable of nitrate utilisation, nitrate is rapidly – often within some minutes - absorbed and concentrated by a factor of 10 in the saliva. In the oral cavity, anaerobes will utilise nitrate as a terminal respiratory electron acceptor, and nitrite will be formed.

A further transformation of nitrite to nitric oxide may be partly microbial partly non-microbial. The latter will take place in the stomach at low pH.

Recently, comparative studies in germ-free and conventional rats have highlighted the important role of the oro-intestinal flora. In some ongoing studies, we are screening probiotic strains for their capabilities of forming nitric oxide.

LIFE IS NOT POSSIBLE WITHOUT BACTERIA (*L. PASTEUR*)

Background

Nitrogen (N) is an essential part of all life. As the gas dinitrogen (N_2) it constitutes the major part of the air. However, only some soil microorganisms are the only organisms capable - by dinitrogen fixation - of transforming atmospheric nitrogen into organic material. In the other end of the chain, denitrification is an important biological mechanism for the return of fixed nitrogen to the atmosphere. Again, this process is carried out by microorganisms, and involved four consecutive reactions in which nitrate is reduced to dinitrogen gas by the metallo-enzymes, nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase. Although many molecular aspects of microbial denitrification recently have been elucidated, the eco-physiology of this important process is hardly understood (*Van de Pas-Schoonen*, 2004).

Whatsoever, Pasteur was right. In a broader scale: Life on Earth is not possible without bacteria.

Excretion and recycling of nitrogen

All multi-cellular organisms have mechanisms for excretion – and recycling – of nitrogen. The three major excretion products are ammonium (amphibian), uric acid (birds) and urea (mammals). However, it should be taken into consideration that all three compounds are found in all macroorganisms.

Under physiological conditions, urea is the major excretion product in mammals. In all mammals, a considerable part is excreted into the gastrointestinal tract, especially the stomach. This gastrointestinal excretion is strongly species-related. In man, it may account for around 20-25%, whereas the gastric excretion in camels may reach 90%.

It seems reasonable to assume that this extra-renal excretion has several functions. In camels, the high excretion gives ruminant microorganisms enough nitrogen for a proper digestion of cellulose-rich desert plants. Additionally, it will be less urea for renal excretion, thereby saving valuable water for the camel.

Many microorganisms in the GI tract, as most of the *Helicobacter* species, many enterobacteria and lactobacilli, including many probiotic strains, produce ureases. On the other hand, endogenous mammalian ureases do not exist. When urea is excreted into the GI tract it will immediately be utilised by the microorganisms for their own metabolism. The breakdown of urea will go through production of ammonium. However, it should be taken into consideration that the toxicity of ammonium is pH dependent. The ammonium thus formed may influence upon the local pH, thereby also influencing upon the ecological balance. Under physiological conditions the microbes themselves will utilise the formed ammonium. If this microbial utilisation of urea takes place in the upper part, the microbes may undergo lyses further down in the GI tract, thereby supplying the macroorganisms with N-containing material, as amino acids, peptides, nucleotides, etc.

The nitrate-nitrite-nitric oxide pathway

Nitrate is an important anion in the GI tract. Vegetables are the main dietary source of nitrate, accounting for up to 2/3 of the dietary intake. Once ingested, nitrate is rapidly absorbed from the gastrointestinal tract and mixed with endogenously produced nitrate. The latter is mainly coming from the L-arginine NO pathway (*Leaf* et al., 1989). The absorption of orally given nitrate is very rapid, i.e. within some few minutes after ingestion. The salivary glands are actively “extracting” nitrate from plasma, the resulting salivary nitrate concentration can easily be at least 10 times higher than the concentration in plasma.

Salivary nitrate is rapidly reduced to nitrite by commensal bacteria in the oral cavity, and at least three types of nitrate reductases may catalyse this reduction (*Potter* et al., 2001). All three types are molybdoproteins and some bacteria, as *Paracoccus pantotrophus,* possess all three (*Richardson* et al., 2001).

During more than half a century, nitrate has gained a bad reputation, preliminary due to its assumed association with development of some types of cancer (*Fraser* et al., 1980) and methaemoglobinaemia. The last condition mostly affects infants up to one year of age and is caused by oxidation of nitrite or nitric oxide, of haemoglobin in red blood cells to an abnormal form, methaemoglobulin, that cannot bind or transport oxygen. In most countries, regulatory authorities have expended great efforts to minimise dietary intake of nitrate. They may have forgotten that possible deleterious effects of human health are not primarily related to the nitrate ion itself. That ion has a remarkable low toxicity. They may also have forgotten that on a typical Western diet, endogenous formed nitrate may account for as much as 50% of the total daily nitrate load. In fact, there is still no clear evidence for a link between nitrate intake and gastric cancer in humans. In fact, some studies show either no relationship or even an inverse relationship between a high intake of nitrate and the occurrence of gastric cancer (*McKnight*, 1999).

When salivary nitrite is reaching the stomach, it is rapidly converted no nitric oxide by non-enzymatic mechanisms. Once formed, nitric oxide exhibits a variety of biological activities and is assumed to be involved in regulation of blood flow, gut motility, secretory and immunological functions (*Bjorne* et al., 2004, *Herulf* et al., 1999), and it may be toxic to several bacteria and viruses (*Duncan*, 1997)

Comparative studies in germfree (GF) and conventional (Conv) rats

In a recent, comparative study (*Sobko* et al., 2004) in GF and Conv rats we found that the concentration of NO in Conv rats differed profoundly along the GI tract; 4000 ppm, 20 ppm, 200 ppm, 20 ppm in the stomach, small intestine and colon, respectively. Gastric NO increased greatly after a nitrate load. Concentration of NO was significantly lower in all 4 compartments in GF rats (31 ppm, 10 ppm, 9 ppm, 13 ppm in the stomach, small intestine, coecum and colon, respectively), and gastric NO remained low after a nitrate load. Giving a NOS inhibitor to Conv rats, we could reduce the NO concentration in the colon, without affecting NO in the stomach and in the coecum.

In a previous comparative study in GF and Conv rats we found no significant difference in NO in exhaled air (*Persson* et al., 1994). Additionally, in another study in newborn babies we found that nitric oxide was present in faeces first after some days (*Sobko* et al., 2005). In an ongoing study we are investigating nitrate and nitrite reducing capacity in strains, including probiotics, belonging to species known to be present in the gastrointestinal flora under physiological conditions.

Taken together, our results clearly show that when microbes and nitrate are present in the same compartment at the same time, the possibility exists for an increased luminal concentration of NO. The physiological and patho-physiological consequences of this microbial production should be further investigated.

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