

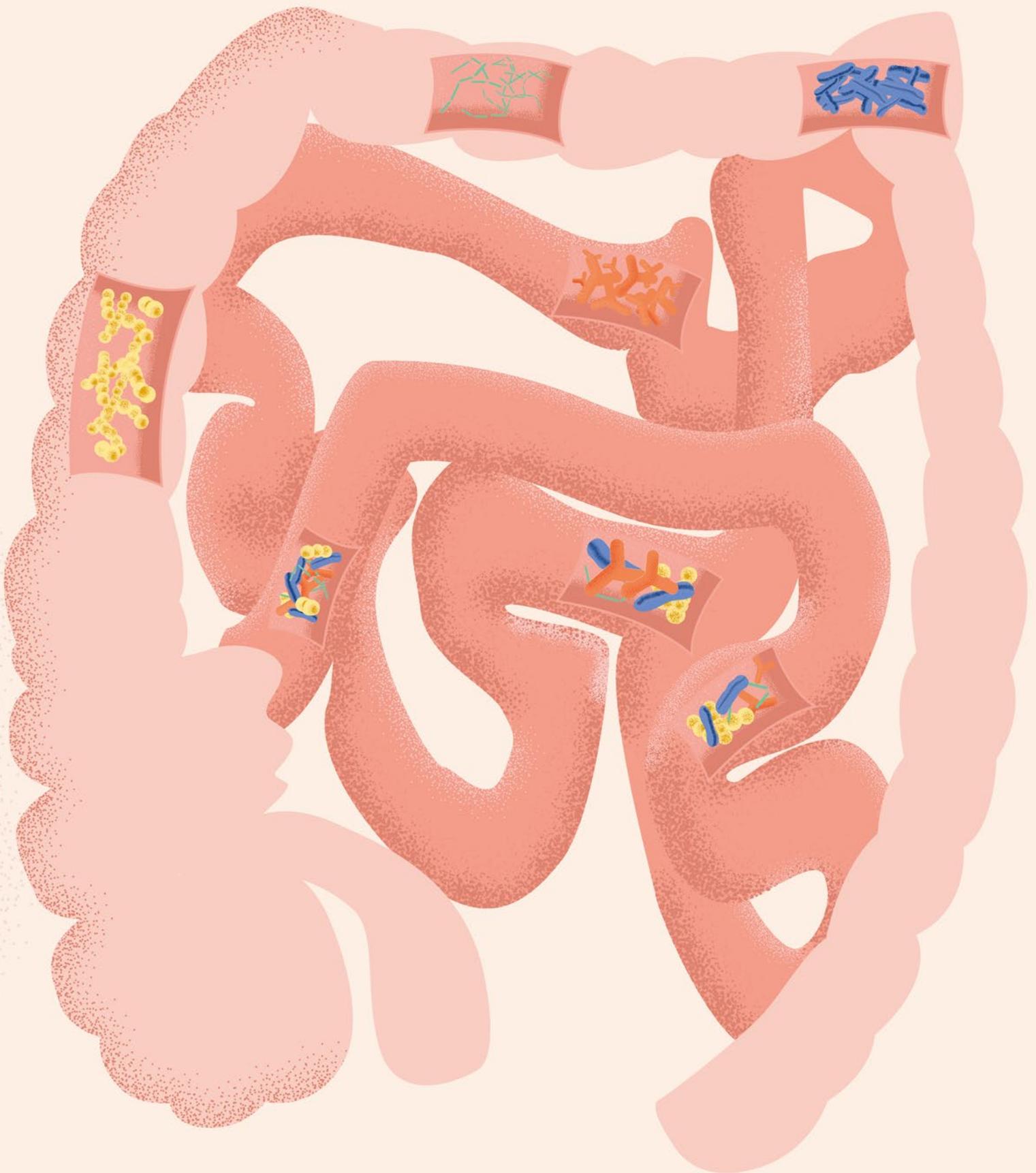
Microbiome

– The mysterious ecosystem in our gut

Fast forward to 2040: A small stool sample will be all it takes to tell if someone is susceptible to certain diseases. You can then take the appropriate preventive action to avoid contracting them, or at least provide better treatment. Unfortunately, however, we have not yet advanced to that stage. But researchers are optimistic that in the next few years, they will be able to detect the characteristic signatures of many diseases in our intestinal bacteria. TUM Professor Dirk Haller and his team are working flat out to achieve it.

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The human intestines are populated by a dense network of microorganisms, the microbiome. It is dominated by four phyla of bacteria forming up to 200–300 different species in an average adult. In a healthy human, these are in equilibrium.

Each and every one of us, whether clean or not, is populated by about 100 trillion bacteria – on our skin, in our saliva, behind our ears or in the crook of our arm. Most of these microorganisms, however, are to be found in the intestines, adding up to one kilogram to our weight. Their community, referred to as the microbiome – gut or intestinal flora in somewhat obsolete terms – consists of a blend of bacteria, some physiologically beneficial, and some pathogenic, depending on the family of bacteria to which they belong. Only if the latter are kept under control are human beings healthy, as then the balance is maintained. In that case, their microbiome supplies an entire range of enzymes which help to digest food. It also produces vitamins and short-chain fatty acids. These are important tasks which ensure that food not digested in the small intestine can continue to be exploited regardless of whether we are eating a juicy steak or a bowl of salad.

In addition, researchers are identifying an ever-increasing number of functions performed by the intestinal microbiome. First and foremost, our immune system is apparently existentially dependent on the bacteria. They act as a kind of sparring partner, giving it the confidence to distinguish dangerous pathogens from harmless constituents of our food. After all, the food we partake of contains numerous foreign germs and pathogens. The wall of the intestine must keep them at bay, i.e. erect a barrier, while on the other hand, it must let through the elements in our food containing energy so that they can reach the bloodstream. If something goes wrong here, we run the risk of developing allergies or autoimmune diseases.

How many species of bacteria are there in our intestines?

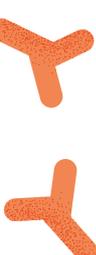
There are around 1,500 species which can populate the intestines. The average European has around 200–300. But a sick person may only have 30–50 (Crohn's disease). And if there are complications after a stem cell transplant (graft-versus-host disease), the microbiome may under certain circumstances shrink to one strain. This is highly dangerous.

When the system tips

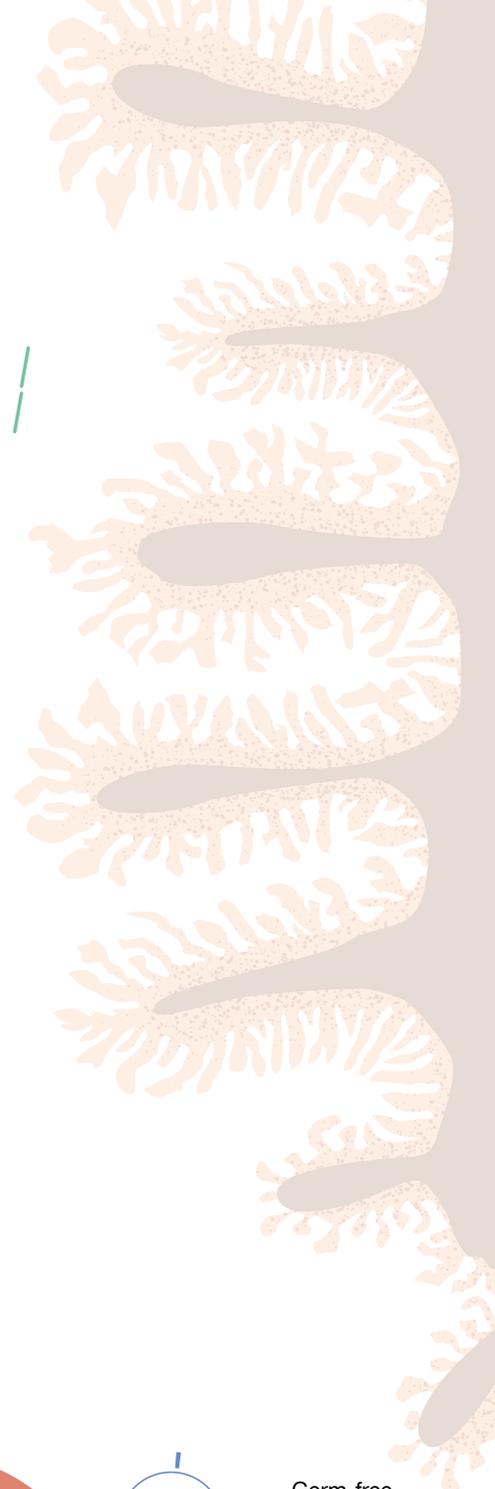
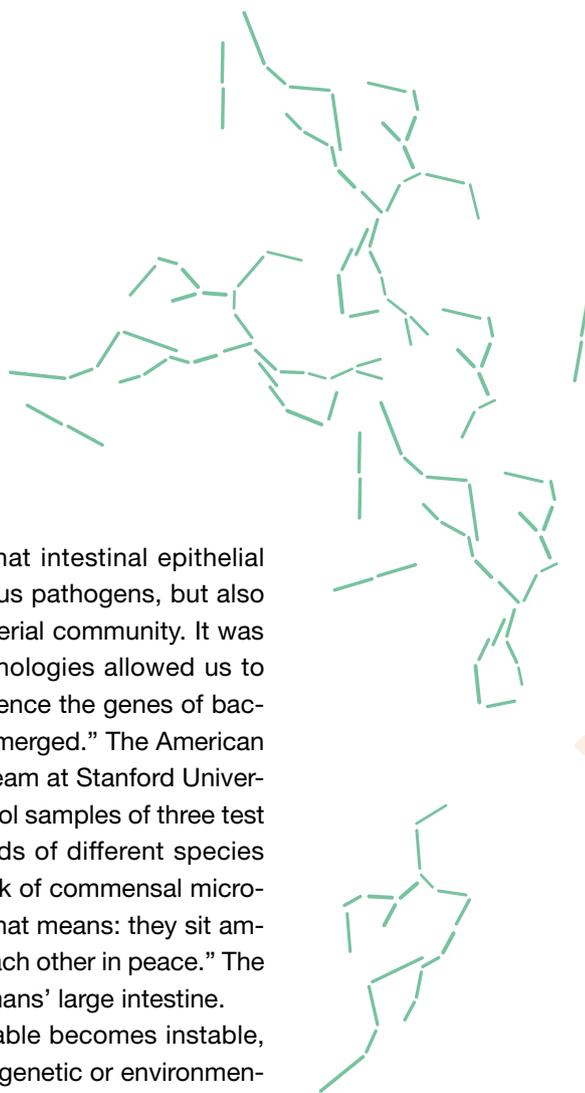
This is precisely the situation that Professor Dirk Haller takes as his starting point. Haller heads up the Chair for Nutrition and Immunology at TUM's Weihenstephan School of Life Sciences, as well as the Institute for Food & Health (ZIEL) which is also located there. Since his Ph.D. thesis, the 51-year-old nutritional scientist and microbiologist has been fascinated by the complex collaboration between the microbiome and its host. "Back then, a good 20 years ago, hardly anyone was interested in the subject," he remembers. "Initially, I myself came from the area of food microbiology, i.e. nutritional science, and we asked ourselves how probiotic bacteria in the gut communicate with us.

"Today, we are in more or less the same situation with the microbiome as we were 20 years ago with the human genome."

Dirk Haller



Here we challenged the dogma that intestinal epithelial cells not only perceive the infectious pathogens, but also the harmless members of the bacterial community. It was only around 2005 when new technologies allowed us to quickly and comprehensively sequence the genes of bacteria, that a new field of research emerged.” The American scientist Paul B. Eckburg and his team at Stanford University analyzed the bacteria in the stool samples of three test subjects and realized that hundreds of different species were peacefully coexisting. “We talk of commensal microorganisms,” Haller explains, “and that means: they sit amicably around the table and leave each other in peace.” The table in this case is the food in humans’ large intestine. Sometimes, however, this round table becomes unstable, and certain bacteria – triggered by genetic or environmental factors – gain the upper hand. The system threatens to overturn, resulting in the symbiosis becoming a dysbiosis. Something of this nature can occur, for example, when you take antibiotics, resulting in infections with *Clostridium difficile* bacteria. These spore-forming organisms occupy the niche cleared by the antibiotics and multiply quickly. They produce toxins and frequently cause life-threatening diarrhea. When and how dysbioses cause intestinal diseases and how they can be prevented are among the research topics which Dirk Haller and his team are working on. ▶

Germ-free experimental animals



Crohn's disease sufferers



Average European

There are around 1,500 species of bacteria which can populate the intestines; the average European has around 200–300. Crohn's disease patients only have 30–50 left. For experimental purposes, mice are bred entirely free of intestinal bacteria.



In the meantime, the scientist has succeeded in firmly establishing microbiome research in Germany: The German Research Foundation (DFG) has just set up a separate field of research (SFB 1371), with Haller as the spokesperson. “It’s a matter of the functional relevance of the microbiome in the digestive tract,” he says. “We want to find answers to the following questions. When is there a causal link between a change in this microbial ecosystem and an illness? Can this be used for diagnoses? Or for prognoses? Or for therapeutic purposes?”

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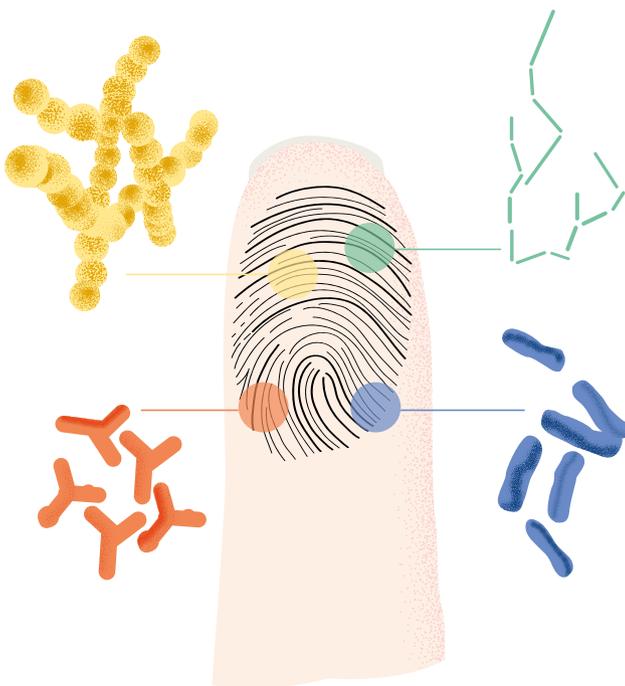
Dirk Haller

New technology has helped the field to flourish

Researchers are pursuing three strategies to clarify such questions.

- › First, they conduct causal examinations on mice and transfer the insights gained to humans.
- › Second, they analyze the microbial ecosystem in the human gut using high-throughput sequencing technologies.
- › Third, in their studies on humans, they search for interventions which can prevent or treat diseases.

The progress of science in the last 15 years comes to their aid here. Research in this field rapidly picked up pace after 2005, the number of publications shot up and ten years later, the microbiomes of thousands of test subjects had already been analyzed. While this led to a huge increase in data, the analysis on its own was not sufficient to draw conclusions with regard to cause and effect.

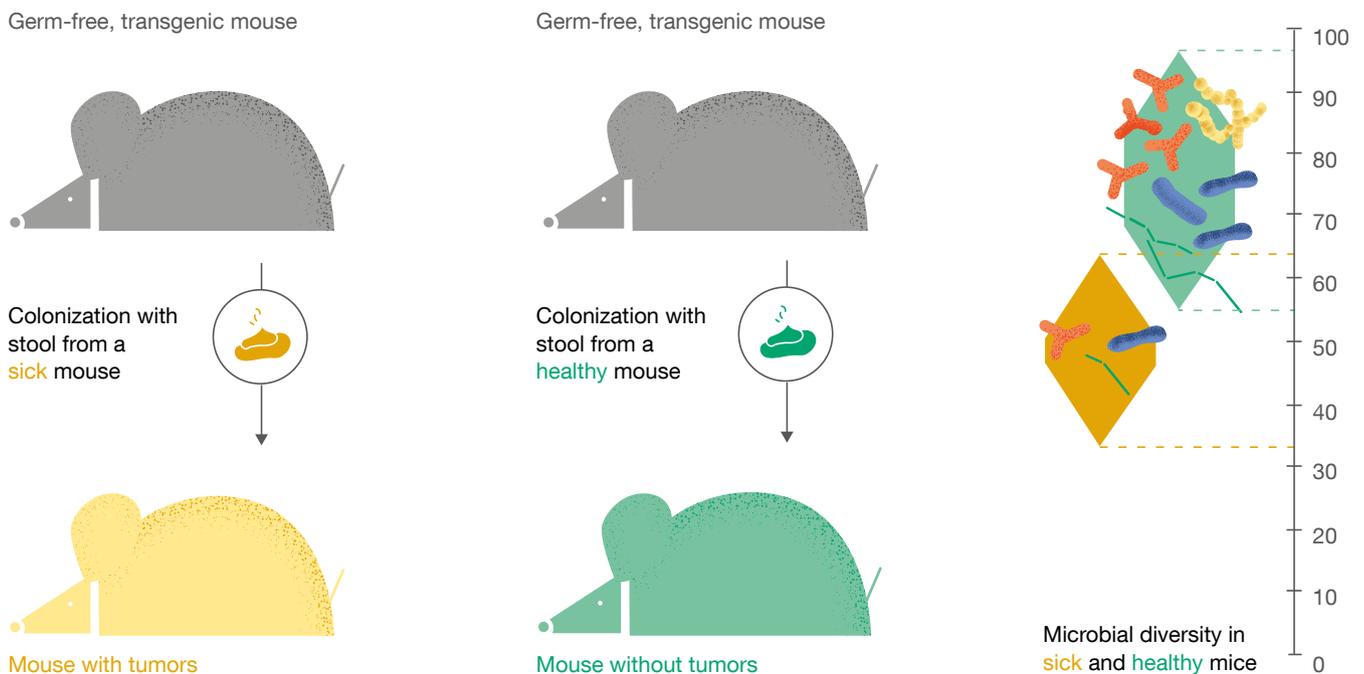


In 2018, for example, a Chinese research group which had examined stool samples of 7,009 persons from 14 districts was amazed by the differences in the bacterial composition between individuals and the major role that lifestyle played. However, they were unable to find what they were primarily looking for, namely a uniform pattern that might point to a general tendency to metabolic disorders such as diabetes. They were only able to detect a regional distribution of individual differences. One central conclusion is that we differ markedly from each other with respect to our microbiomes. The basic finding has now been confirmed that we all have a completely individual fingerprint in our intestines with respect to the composition of the bacteria colony. We can find a large degree of overlap between individuals, but if we look at the level of individual bacterial strains, our microbiomes differ totally. And the differences are so marked that they could even be used forensically, in other words to identify any person by their stool sample. Nevertheless, there are still many more unanswered questions than problems solved. “Today we are in more or less the same situation with the microbiome as we were 20

years ago with the human genome,” Haller says. “Back then, we had high hopes that we would soon be able to recognize every disease genetically. This did not materialize, however. In diagnostic terms, success has been decidedly modest. Although we have a much better understanding now, when it comes to applying our findings, we are still in the starting blocks.” For this reason, he and his colleagues are now leveraging all the opportunities offered by modern analysis and bioinformatics.

The animal model as a template

For example, Haller has established his own mouse-breeding center and sterile mouse husbandry at his institute. Around 1,200 mice live in 25 isolators with sterile air and sterile food, in a germ-free environment from birth. They also have no bacteria whatsoever in their gut, i.e. no microbiome, and are ideally suited for researching the effect of certain implanted bacteria or groups of bacteria in their intestines. ▶



At Dirk Haller’s Institute, mice are bred in a germ-free environment. As they do not have any intestinal bacteria of their own, they react very specifically to the transfer of stool samples from other mice. For example, it can be shown that certain microbiomes can transmit bowel cancer. The diversity of intestinal bacteria is significantly higher in healthy mice than in sick ones.

The microbial diversity in human intestines – symbolized here by colors – has steadily decreased the more people have adopted an industrial lifestyle.



“In order to distinguish which bacterial changes make us susceptible to illness from the heterogeneity and individual diversity of people, we translate this into animal models,” the researcher explains. “For example, we take sterile animals which are genetically susceptible to a disease. If they then do not become ill in spite of their predisposition, this will probably be due to the lack of bacteria.” Conversely, a particular microbiome can make the mice ill if it is transplanted – representing an initial step towards proving causality. Stool transplants both between mice and between man and mouse play a major role in such research. In the past, this procedure was also applied to humans, for example in the treatment of life-threatening *Clostridium difficile* infections. Taking countermeasures with a transplanted, healthy, rich ecosystem was successful in around 90% of patients. Otherwise conventional medicine only has the option of prescribing a further antibiotic. But only 20–30% respond to this treatment.

After a death in the USA following a stool transplant, authorities in the EU are now imposing strict requirements. Fecal transplants are only allowed in human medicine for therapeutic use in accordance with Section 2(1) of the Medicines Act. For applications, the material to be transplanted has to be prepared under the direct, technical supervision of the doctor treating the patient and reported to the responsible state monitoring authorities.

Are changes to the microbiome cause or effect?

“We and other groups are currently conducting a world-wide search for cause and effect relationships between microbiome and disease,” Dirk Haller explains. “At the moment, many diseases, starting with dementia and autism and on to cirrhosis of the liver, colon cancer, Crohn’s disease, ulcerative colitis and diabetes are being traced back to the microbiome. And these diseases are indeed associated with changes to the microbiome. The question arises, however, as to whether these changes are the cause or are they perhaps only one effect of the disease?” This chicken and egg problem is occupying him and his staff, primarily in the case of chronic intestinal diseases.

It was definitely established that the microbiome is markedly altered in such patients. As this probably also applies to diabetes patients, the Weihenstephan researchers are also collaborating in the Augsburg cohort study KORA.



Nutritional scientist Dirk Haller heads the Chair for Nutrition and Immunology at the Weihenstephan School of Life Sciences.

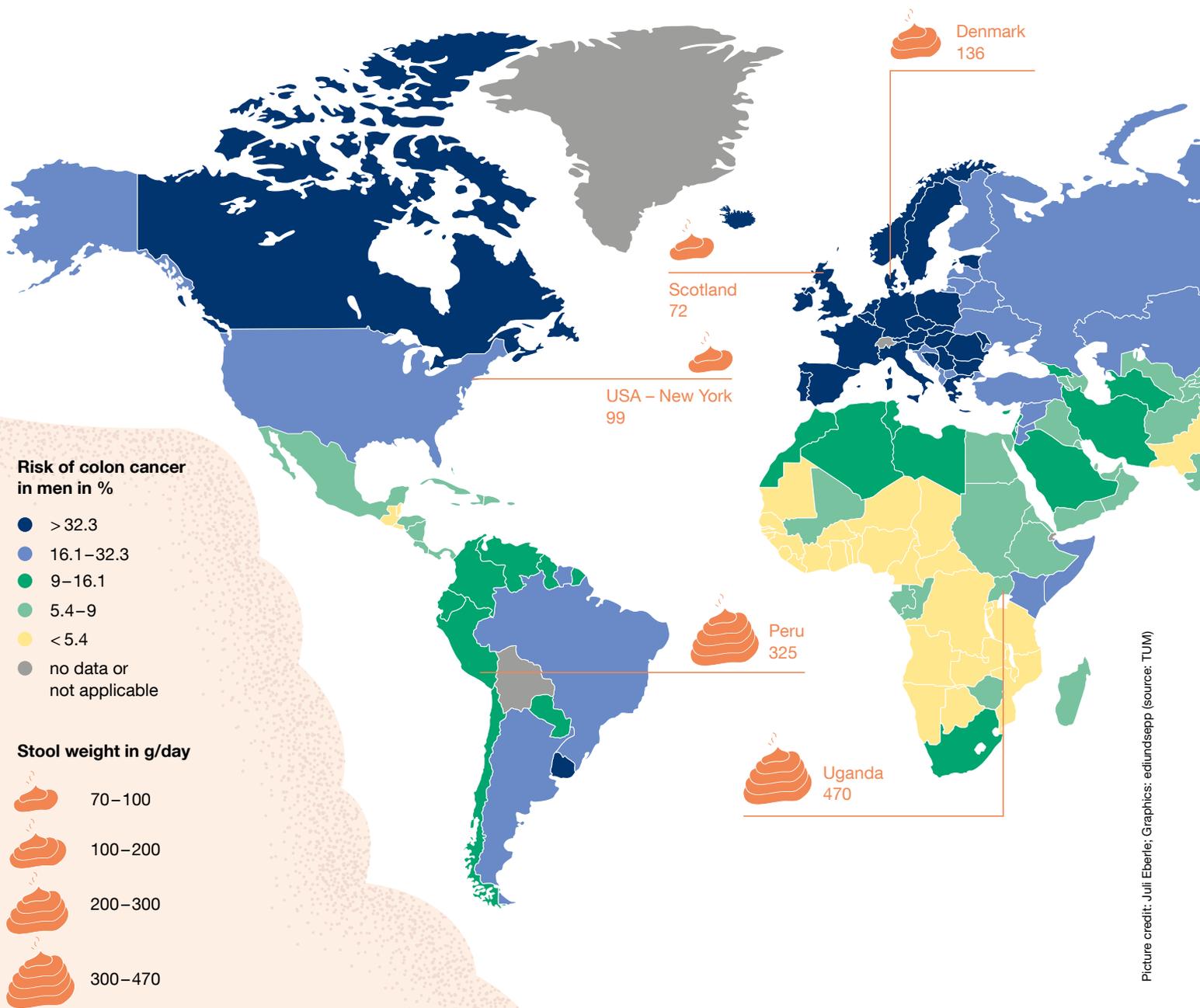
They examined stool samples from 2,500 subjects to find out whether disease signatures for type 2 diabetes can be found. Surprisingly, they found circadian rhythmicity in the microbiome and identified a diagnostically relevant risk profile of arrhythmic bacteria in patients with type 2 diabetes. The scientists analyzed high-throughput sequencing data and applied Artificial Intelligence to identify populations at risk for type 2 diabetes. The key questions for a successful clinical application are the following. Are microbiome signatures capable of predicting the future onset of

disease? And, are we able to change disease-conditioning microbiomes?

While chronic inflammatory intestinal diseases didn't even exist in many still under-industrialized countries until recently, statisticians are now recording a sharp increase. This is exemplified in Hong Kong. "There were no incidences of Crohn's disease there," Dirk Haller says. "But in the last 15 years, the frequency of the disease has shot up, i.e. in precisely the period in which the Hong Kong lifestyle has transitioned from traditional to highly industrialized. ▶

Other epidemiological surveys clearly reveal the following: “Obesity is usually associated with chronic inflammation in the same way as type 2 diabetes. This means modern lifestyle makes us somewhat chronically prone to inflammation even if on a different scale if you compare obesity with Crohn’s disease,” Haller states. “At some point – figuratively speaking – a fuse blows in the system, depending on an individual’s genetic susceptibility, and then the disease escalates.”

The following proposition could be derived from these findings: Industrialized lifestyles entail an altered microbiome, and its interaction with the host’s immune system engenders the disease. Mere statistics, however, are not sufficient to prove causality, and for that reason, a functional understanding of the microbiome needs to complement the computational analyses in order to achieve the next level of knowledge about this complex ecosystem. We need to answer the following questions: What characterizes an individual microbiome and why do patients fall ill? What microbial interaction is able to trigger a chronically inflammatory intestinal disease or even turn it into cancer? And is there a way to counteract this effect therapeutically?



Picture credit: Jüli Eberle; Graphics: edlundsepp (source: TUM)

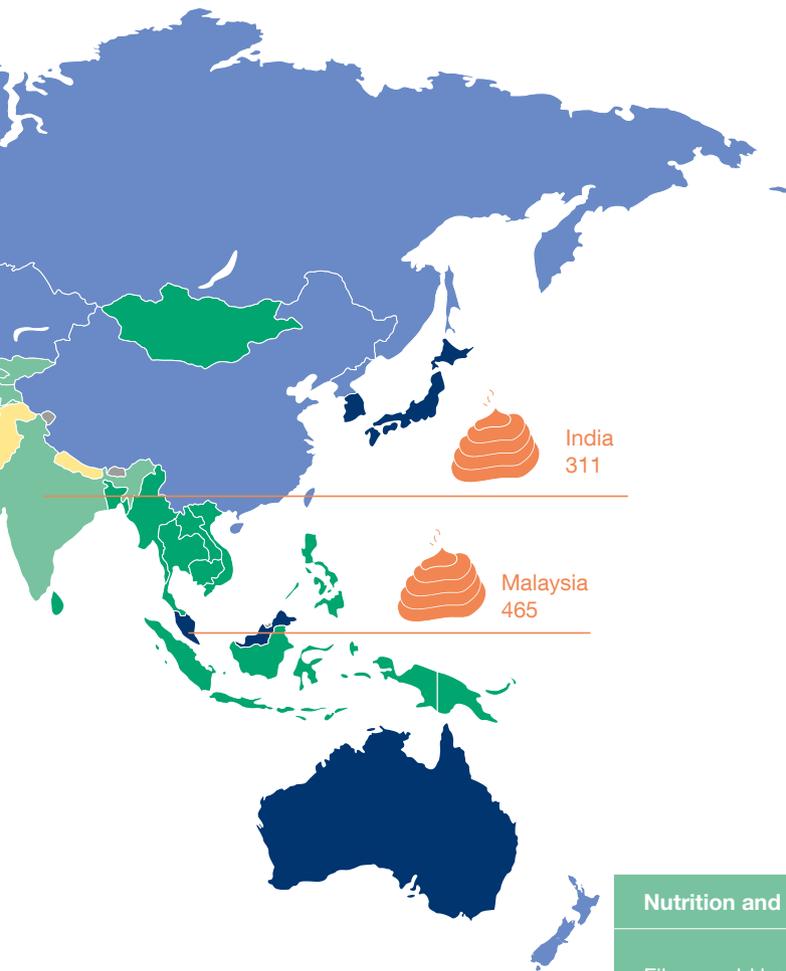
Dirk Haller summarizes the state of play: “There is a lot of correlation, but scarcely any causality. I am convinced it will take another 20 years before we have identified all the causalities and know for sure whether the microbiome is just a symptom or the cause of diseases.” Nevertheless, he sees a bright future for his discipline. “The biology of the microbiome will have a definitive effect on the science of the 21st century, of that I am sure. After this special field of research has finished in twelve years’ time, we want to be able to answer the following question. In which diseases does the microbiome play a role, and in which not, and if it does, what precisely is that role?”

■ *Brigitte Röthlein*



Prof. Dirk Haller

The researcher was born in 1968 and studied food technology and nutritional science at the University of Hohenheim, obtaining a degree in both. Haller then took a doctorate in microbiology and immunology on the subject of “Modulation of the immune response of non-pathogenic bacteria”. After staging posts with Nestlé in Lausanne and at the University of North Carolina, he returned to TUM with an Emmy-Noether group funded by the German Research Foundation where he has held the Chair for Nutrition and Immunology at the Weihenstephan School of Life Sciences since 2008.



Nutrition and the microbiome

Fiber could be one key to a rich microbiome and intestinal health. Central Africans, for example, have an extremely fiber-rich diet. They eat around 70 grams of fiber per day. In Central Europe, we consume around 10-20 grams a day.

