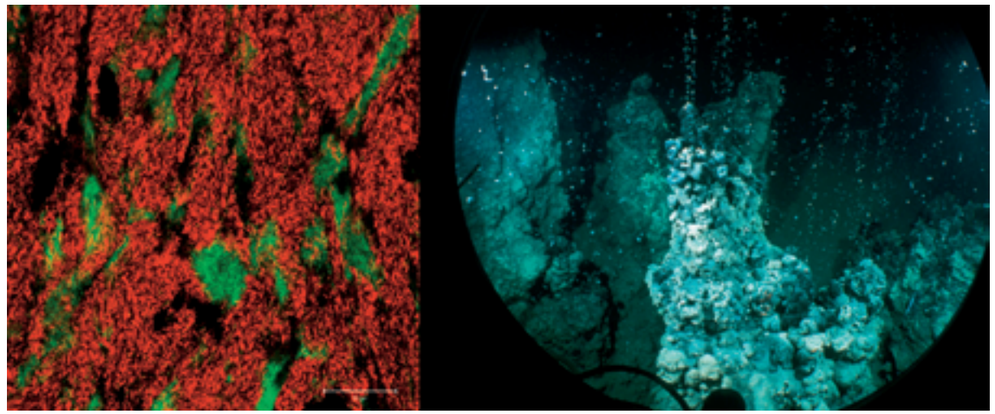
RESEARCH in Brief



This thin section of a reef in the Black Sea was doubly stained for the epifloures-cence microscopy: with both a red-fluorescent RNA-probe, targeted against a specific group of archaea, and a green-fluo-rescent RNA-probe that shows a specific group of sulfate-reduc-ing bacteria. The white bar is equivalent to one fiftieth of a mil-limetre.

The view from the submersible JAGO shows the bacteria reef surrounded by gas bubbles. Some of the reef structures are four metres high and one meter wide. They comprise al-most exclusively of methane-con-suming microor-ganisms, as well as carbonate precipi-tation produced from the conver-sion of methane.

PHOTOS: MPI FOR MARINE MICROBIOLOGY/GHOSTDABS, UNIVERSITY OF HAMBURG

MARINE MICROBIOLOGY

Bacteria Are Master Builders

Gigantic reefs, measuring up to four metres in height and made up of micro-organisms which live on methane have been discovered by scientists from the Max Planck Insti-tute for Marine Microbiology in Bremen, the University of Hamburg and the Alfred We-gener Institute for Polar and Marine Research (AWI) in the coastal waters of the Black Sea (SCIENCE, 9 August 2002). The mats mainly consist of densely aggregated living ar-chaea and sulfate-reducing bacteria which symbiotically produce porous reef struc-tures made from calcium carbonate as well as substan-tial quantities of cell bio-mass. This discovery is of fundamental significance to our understanding of earlier periods in the Earth's history and of the origins of the biosphere.

The view from the submersible JAGO looks out onto a land-scape full of columns, hillocks, nodules and other reef struc-tures and bears very little re-semblance to the image of a small section of the reef mag-nified 1,000 times under the microscope, which shows a close symbiosis of two differ-ent cell types – bacteria and archaebacteria. However, these single-celled organisms, which grow no bigger than one thou-sandth of a millimetre, are able to convert enormous quantities of methane into carbon diox-ide (CO_2), thereby constructing huge deposits of organic matter and carbonate.

For more than 30 years, scien-tists throughout the world have attempted to find mi-croorganisms which are capa-ble of converting methane in the absence of oxygen. Methane is an essential com-ponent of natural gas, which we use as fossil fuel. It is also an important greenhouse gas that is produced in large quan-tities on land – particularly in rice paddies and the rumen of cows – as well as deep under the seabed in the oceans. Just two years ago, a research group made up of microbiolo-gists, molecular ecologists and biogeochemists from the Max Planck Institute for Marine Bi-ology in Bremen succeeded for the very first time in tracking down this type of life form above gas hydrate deposits in the sea (MAX PLANCK RESEARCH 4/2000, Page 5 ff.). This finding also involved a symbiosis of archaea and bacteria which occur together in tiny cell clumps in their thousands in methane-rich seafloors and have in the meantime been found throughout the world in gas-enriched locations.

The water at the bottom of the Black Sea – the largest oxygen-free marine basin in the world – also contains adequate sup-plies of methane. With the help of the submersible JAGO, the researchers have now discov-ered an enormous reef to the west of the Krim Peninsula at a water depth of 230 meters. Gas bubbles continuously flow through the structures, which can measure up to four metres in height and one meter in width. These structures consist of dense microbial mats that are internally supported by means of lime-like carbonate precipitations. Like hydrogen sulphide, the carbonate occurs as a waste product from the oxidation of methane with sul-fate which exists in abundant quantities in the seawater.

The microorganisms that are responsible for creating the reef in the Black Sea are rela-tives of the small cell clumps made up of archaea and bacte-ria discovered two years ago above gas hydrate.

The inhabitants of the Black Sea are also capable of con-verting large quantities of methane with sulfate and of using it as a source of carbon for their growth. This is shown by the investigations carried out by the researchers at the University of Hamburg which were able to provide evidence of a high content of carbon from the methane in typical biomass components of ar-chaea and bacteria. The ability of these microbial mats to convert enormous quantities of methane and sulfate was already measured on board the Russian research ship RV Lo-gachev by Katja Nauhaus and Tina Treude, PhD candidates at the Max Planck Institute for Marine Microbiology. Dr. Katrin Knittel and Dr. Armin Gieseke, postdocs who also work at the Max Planck Institute, helped to solve the mystery surrounding the identity of the microorganisms. By directly staining the cells using specific genetic probes, they were able to show that it is those micro-colonies of methane-consum-ing archaea and sulfate-re-ducing bacteria which form dense mats permeated by small veins. It is likely that these tiny channels assist the ex-change of nutrients and meta-bolic products and flow into larger hollows and spaces in the calcareous interior of the reef structures.

But what have these gigantic bacteria reefs got to do with the Earth's history? They repre-sent the first living proof that huge accumulations of organic material could have developed even without oxygen and plant biomass in a chemosyn-thetic manner. For a long time scientists have been discussing whether or not methane could have played a role as nutrient and energy carrier in the early history of life several billion years ago.

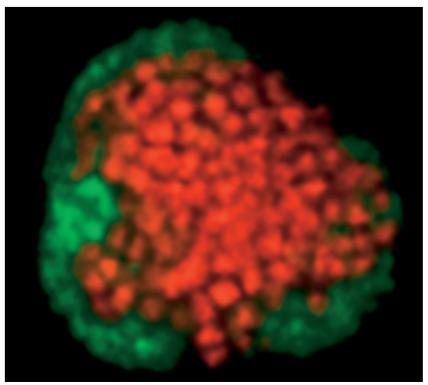
The prevailing theory still implies that only oxygen-con-suming microorganisms were able to use methane efficiently. However, oxygen originated after the development and spread of plant life, as we know it today. The AWI scien-tist, Antje Boetius, co-author of the SCIENCE study, therefore makes the following conjec-ture: "Perhaps the original in-habitants of the Earth during a lengthy period of its history were the same kind of microor-ganisms as those we discovered in the Black Sea: a symbiosis of cells which can grow in the absence of oxygen using methane for nutrition."

These microorganisms would provide the missing link in the chain of methane cycling during very early periods of the Earth's history. This methane cycle would then consist of four stages: 1. Bac-terial fixing of carbon dioxide by means of sunlight without oxygen formation (anaerobic photosynthesis); 2. Decompo-sition of photosynthetic bio-mass through fermenting mi-croorganisms; 3. Formation of methane through methano-genic archaea; 4. Consumption of methane in the absence of oxygen by methane-consuming (methanotropic) archaea.

Another biotope which is in-teresting because it remains largely unexplored and in which the conversion of methane plays a large role is the deep biosphere that still shows signs of life at a depth of up to several kilometres below the seafloor.

Further extreme locations for methane-consuming microbes, such as mud volcanoes on the seafloor, permafrost soil in the arctic tundra, or the ice in the polar regions, are being investigated by various re-search groups at the Alfred Wegener Institute for Polar and Marine Research. These and other projects on the glob-al methane cycle are being supported by the German Ministry for Research and the German Research Council within the framework of the current programme "Geotech-nologies – gas hydrates in the Earth's system". This venture was started at the end of 2000 and already in its first year has produced many new dis-coveries on the role of natural gas in the environment. ●

A closely-related symbiosis of methane-consum-ing microorgan-isms. Researchers first discovered the small clumps of archaea bacte-ria (red) and sul-fate-reducing bacteria (green) in sediments con-taining gas hy-drate taken from the continental slope off Oregon (USA).



PHOTOS: MPI FOR MARINE MICROBIOLOGY/GEOMAR

On board the RV Logachev, samples are taken from pieces of the bac-teria reef for the purposes of carry-ing out various experiments on material conver-sion and on the nature of the bacteria and archaea.

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RESEARCH *in Brief*

BIOGEOCHEMISTRY

The Greening of the North

Twenty years of satellite observations have indicated a "greening" trend in northern regions of the northern hemisphere. Scientists at the Max Planck Institute for Biogeochemistry in Jena, led by Prof. Colin Prentice, and from the Potsdam Institute for Climate Impact Research, Lund University, Sweden, Boston University, and the Laboratoire des Sciences du Climat et l'Environnement, Paris, have developed an advanced global ecosystem model showing that this trend can be explained by climatic warming (SCIENCE 31 May 2002).

Some years ago, Ranga Myneni and his colleagues at Boston University published a paper in Nature reporting on a "greening trend" in the boreal regions (taiga forests and tundra). The finding was based on satellite observations, and many scientists were sceptical about it because of problems of instrumental "drift" that could possibly make any observed long-term trend unreliable. The Boston team, however, persisted in their efforts to re-analyse the data, taking into account all possible anomalies. The trend persisted, and it has become clearer with time as new data has come in.

A possible cause of the increased greening of the north was obvious: weather station records from the north have reported a steady warming (by about 0.4 degrees per decade) which is now generally attributed to the increasing greenhouse effect. Warming in the north is expected to produce greening. However, it was not easy to connect the weather station data to the satellite data to find out whether there was a quantitative correlation between the two trends. Although computer-based numerical models of the physical climate have been used for three decades to analyse the possible consequences of increasing concentrations of greenhouse gas, comparable global models of ecosystem dynamics are a much more recent development.

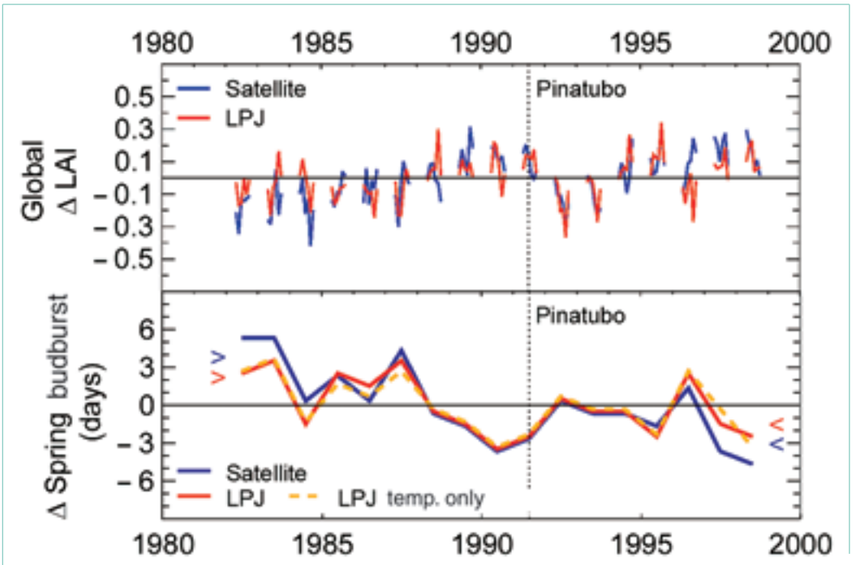
Now, a major activity of the Global Ecology research group at the Max Planck Institute for Biogeochemistry in Jena, under the leadership of Prof. Colin Prentice, is the ongoing development of a leading global terrestrial ecosystem model. The model is called LPJ, after the three research groups (led by Prof. Colin Prentice in Jena, Prof. Martine Sykes in Lund and Prof. Wolfgang Cramer in Potsdam) which participate in the model development consortium. The basic idea of the model is to integrate current knowledge in separate fields (such as plant physiology and biophysics, terrestrial ecology and hydrology), to simulate the interaction of processes with different time constants (minutes to years), and to use all possible sources of information – including satellite observations – to evaluate and refine the model.

The new results published in SCIENCE are the product of a close collaboration between the LPJ consortium and the research group in Boston – spearheaded by Dr. Wolfgang Lucht, a young scientist from the Potsdam Institute for Climate Impact Research who has close links to the remote sensing community. This collaboration made it possible for month by month climate observations from across the entire boreal zone to be used to drive the LPJ model. The scientists discovered that the expected changes in leaf area index (ratio of total leaf area to ground area) during each growing season and (most importantly) from season to season, based on climate date, fitted remarkably well to the satellite data.

Two decades of satellite observations showed that spring has advanced by about a week, and that summer maximum leaf cover has increased as well. The model results showed that these trends correspond quantitatively to what should be expected based on the warming that has occurred. The only plausible explanation for this agreement between two fully independent sources of information is that the remotely sensed trend is real, and that it was caused by the changing climate.

With a model such as LPJ, it is also possible to perform sensitivity tests to isolate the key factor causing a given effect. It was found that the trend was caused entirely by temperature. It was not caused by physiological effects such as quicker plant growth brought about by increased carbon dioxide concentrations or by the increasing rain- and snowfall that has accompanied the increase in temperature.

One major event temporarily interrupted the trend: the 1991 eruption of Mount Pinatubo in the Philippines. The fallout of volcanic aerosol produced a 0.5 to 1 degree average cooling – as well as spectacular sunsets – in northern high latitudes during 1992 to 1993. Again, satellite data had already shown an interruption to the greening trend. And, once again, many scientists were sceptical about the correlation between temperature change and increased greening because the volcanic aerosol itself affects the performance of the space-borne instruments. But in this case, too, the results of the LPJ model showed the same thing as the satellite observations – a short-lived dip in the trend which was promptly resumed after the volcanic dust had settled.



Above: Differences of leaf area index (LAI) in the global boreal zone from the long-term average for each month as shown by satellite data and as simulated by the LPJ model. The leaf area index is the ratio of total leaf area to ground area. Below: Change in the timing of spring budburst in the global boreal zone as differences from the long-term average for each year shown by satellite data and simulated by the LPJ model. The dashed line shows an LPJ model run in which only temperature was varied from year to year.

The Pinatubo eruption had another effect, too, which greatly surprised carbon cycle scientists when it occurred: the rate of increase of carbon dioxide in the atmosphere suddenly slowed down for two years. A full explanation for this (which probably involves temperate and tropical regions as well) has not yet been found. However, it was possible to engage the French team at the Laboratoire des Sciences du Climat et de l'Environnement in Paris which has been using advanced mathematical tools to analyse the small variations in carbon dioxide concentration that are observed between different measurement stations across the globe, in order to infer the changing patterns of regional sources and sinks of carbon dioxide on land and at sea. Their results showed that during the "Pinatubo years", the land at high latitudes was taking up a great deal more carbon dioxide than usual.

The LPJ model results showed exactly the same thing. Even though the growing season was shortened and photosynthesis reduced, the cooler growing season meant slower decomposition of organic matter in the soil. This was the dominant effect of cooling for the net carbon exchange of the land in high latitudes, and it contributed to the slowing of the carbon dioxide growth rate on a global scale.

These results represent a "coming of age" for ecosystem modelling. They clearly demonstrate the value of collaboration between observational and theoretical communities. Much ecosystem modelling up to now has been aimed at forecasting the impacts of future climate change, but in this aspect the different models show large differences. Thus, models need better evaluation, and the only way to do this is by making systematic comparisons between model predictions and observations referring to the present and past. The results have implications beyond academia. The researchers have shown that the biosphere is indeed changing due to a climate change that most likely is a direct consequence of human activities (principally in the industrialized countries). The warming and greening of high latitudes, if they continue, will have mixed effects from the point of view of the societies involved. The potential for forestry and agriculture in northern Canada and Siberia will improve, but Arctic ecosystems and species, and the indigenous cultures that depend on them, will be threatened by the invasion of plant and animal species from the South. ●

FIG.: MPI FOR BIOGEOCHEMISTRY

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BIOLOGICAL CYBERNETICS

Memories in Living Colour

Fig. 1: The test images – presented in colour and in black and white.

If a picture is worth a thousand words, then a picture with colours is worth even more. Pictures with natural colours help us to store images more efficiently than black and white scenes and as a result to remember them better. This has been demonstrated in the work carried out by Dr. Felix Wichmann from the Max Planck Institute for Biological Cybernetics in Tübingen, and Karl Gegenfurtner, formerly from the Max Planck Institute and now Professor at the Justus Liebig University in Giessen (JOURNAL OF EXPERIMENTAL PSYCHOLOGY: LEARNING, MEMORY AND COGNITION, Vol. 28, No. 3, May 2002).

As a result of investigations carried out into visual perception, scientists had already been aware for a long time that nerve cells of the visual cortex in the brain of primates reacted in different ways to light of varying wavelengths. Psychophysical experiments on colour perception had already been carried out by Newton and Goethe. However, despite the numerous tests that were undertaken and the multitude of findings obtained on the human powers of subtle colour discernment or on the constancy of colour in different lighting conditions, results which suggested a corresponding role of colour in visual cognition – i.e. the recognition of objects – were rare: whilst colour is attractive to look at, it does not appear as such to be important for object recognition.

In a series of five experiments, the scientists in Tübingen have now demonstrated that colour has a detectable influence on the human memory for natural scenes. In the first experiment, participants were presented with images belonging to different categories: green meadows and forests from the Tübingen area, flowers, the more barren landscapes of Utah, as well as urban scenes showing cars, houses and people. The images were either in colour or in black and white (Figure 1) – participants were able to remember the colour photos significantly better than they remembered the black and white images. In order to rule out whether colour's built-in appeal caused the advantage by grabbing participants' attention or by creating a higher internal contrast than that of black and white images, the scientists carried out experimental variations – always with the same result. In a further experiment, the researchers showed "false colour images". These photos, shown for example in Figure 2, have the same brightness at each point as natural colour images. Result: our brain deals with false colour images in the same way as black and white images. It appears as if the human memory system has become tuned, by evolution and development, to the colours of the natural world. If images deviate too greatly from the natural norm, then the memory system clearly does not engage as well. At the same time, the colour advantage of natural images cannot simply be explained by the fact that colour photos contain more information, i.e. more bits, as false colour images contain just as many bits as naturally coloured images. ●

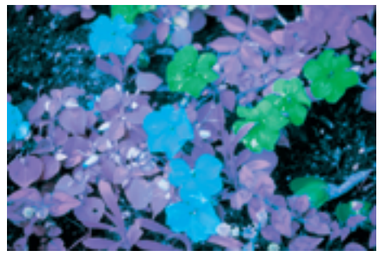

Fig. 2: An example of a false colour image (the photo in Fig. 1, top right, shows the same subject in natural colours).

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PHOTOS: MPI FOR BIOLOGICAL CYBERNETICS.

EXTRATERRESTRIAL PHYSICS

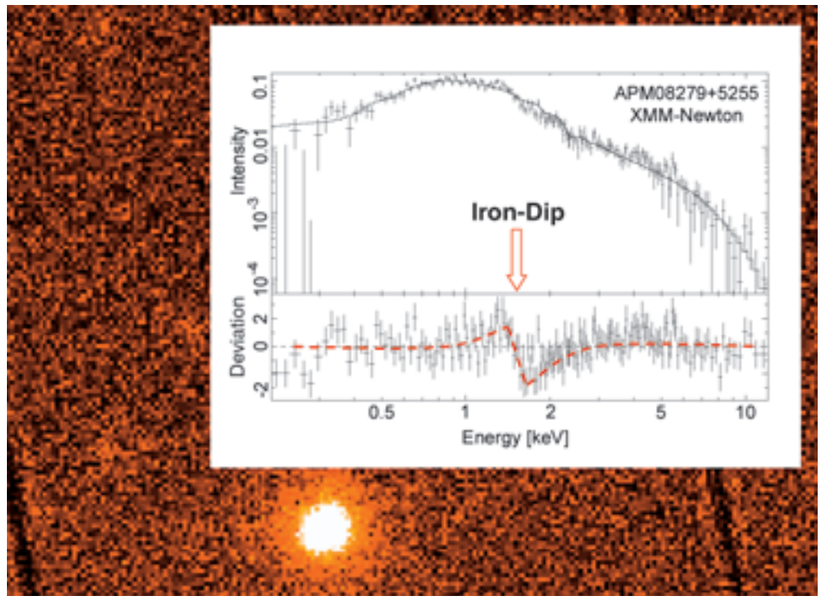
Mysterious Iron Factory in the Early Universe

Where does iron come from? According to astrophysicists, iron is created in the centre of massive stars and is expelled into space once these stars explode as supernovae at the end of their lives. The material then mixes with the interstellar matter and may form new stars and planetary systems. Our solar system was formed after several generations of stars and therefore contains enough heavy elements like iron, oxygen, etc. to form Earth-like planets which can sustain life. Prof. Günther Hasinger and Dr. Stefanie Komossa from the Max Planck Institute for Extraterrestrial Physics in Germany, and Dr. Norbert Schartel from the European Space Agency ESA in Spain, have made a startling discovery: spectral observations carried out with the X-ray observatory XMM-Newton demonstrate that the young quasar APM 08279+5255 contains a three times larger iron fraction than our own solar system. We observe the quasar at a time when the universe was only about 1.5 billion years old; in contrast, our sun was formed 9 billion years after the Big Bang. This is significant in that the centre of this young quasar already contains a larger fraction of iron than our much older solar system. Either there is a previously unknown and much more efficient way of producing iron, or, at the time when the quasar emitted its light, the universe was already older than expected (APJ LETTERS Vol. 573, L77, 10 July 2002).

The quasar APM 08279+5255 is one of the most luminous objects in the universe. Its energy output exceeds that of our sun by more than a quadrillion (10^{15}). This is the only reason why we can still detect intense radiation from this quasar despite its enormous distance. The quasar's luminosity is mainly powered by gaseous matter sucked by a giant super massive black hole at the centre of the quasar. The material becomes super-heated during this process and emits X-rays before it disappears forever into the black hole.

Part of this matter, however, is not swallowed but instead forced out by the intense radiation pressure of the central object. In the case of APM 08279+5255, we are looking down the stream of this outflowing material. In addition to being intrinsically luminous, the quasar's light is further magnified by a so-called gravitational lens. These properties make APM 08279+5255 an excellent "laboratory" in which to study the physical conditions in the early universe and in the immediate vicinity of super massive black holes.

During analysis of the quasar's X-ray light, detected with the European X-ray satellite XMM-Newton, Günther Hasinger,



The "dip" in the spectrum of the quasar APM 08279+5255 (the picture shows a photo taken by XMM-Newton) is caused by the element iron. Similar to the way in which physicians visualize our bones using X-rays – bones appear dark since they are opaque to X-rays – the outflowing iron clouds of APM 08279+5255 are opaque for X-rays which are created at the quasar's centre: at the "absorption energy" characteristic for iron (marked by the red arrow), part of the X-ray light is missing.

FIG. AND PHOTO: ESA/MPI FOR EXTRATERRESTRIAL PHYSICS

Stefanie Komossa, and Norbert Schartel noticed that the material streaming away from the centre of the quasar contains huge amounts of iron. From the "dip" in the quasar spectrum the scientists could determine the amount of iron located in the central region of the quasar, and thus in the early universe. Interestingly, iron appears to be the only element clearly showing up in the spectrum; other elements, like oxygen, are barely detectable. The estimated ratio of iron to oxygen is about three to five times higher than in our solar system.
All the heavy elements of which planets and humans are also comprised were created inside stars billions of years ago. This is also the case for the element iron which is mainly created by a special type of supernova (type I): supernovae are suns at the end of their lives which pass away in giant explosions, blowing the elements produced in their interior out into interstellar space. Some fraction of this "star dust" is used to build new stars, another fraction is ultimately sucked into super massive black holes at the centres of galaxies. However, since stars which pass away as type I supernovae have rather long lifetimes (about one billion years), large quantities of iron in the early universe are quite remarkable.
The iron abundance is of such significance because iron represents a kind of "cosmic clock": all heavy elements – with the exception of the lightest such as hydrogen and helium – were produced after the Big Bang, around fifteen billion years ago, in the interior of stars by the processes described previously. The creation of iron took considerable time: at least 1.5 billion years to produce the metal abundances of our sun. It is therefore highly surprising that an object as young as the quasar APM 08279+5255 already contained a larger fraction of iron than our sun which is much older. Either there is a much more efficient way of producing iron – some kind of "iron factory" – or the universe, at a redshift of z = 4, is already older than previously expected.
What is the meaning of the redshift "z"? The light which astronomers receive from distant objects has been travelling for an extremely long time. Viewing objects at large distances is therefore equivalent to viewing them in the distant past when they were still young. Telescopes thus resemble "time machines". Accessing ever-greater distances in astronomy allows unique insights into the early phases of the universe. During the time the light needs to travel from a distant galaxy to Earth, the whole cosmos is expanding, stretching the light wave. This increases the wavelength of light, equivalent to a "redshift" (z), which is a measure of the distance and therefore the age of a galaxy or a quasar: the higher the z-value of an object, the larger its distance, and the smaller its age. At the distance of APM 08279+5255 (z = 3.91) the universe was at an age of only 1/10 of its present age of 15 billion years; the quasar's light therefore originated in the early days of the cosmos.
The new observations presented here paint an extreme picture of the centre of the quasar APM 08279+5255: there must have been a whole series of supernovae at the quasar's centre to produce the large amount of iron observed. Furthermore, in order to explain the high luminosity of APM 08279+5255 and the huge outflow of matter from its centre, many solar masses of stardust have to be swallowed and partly blown out again, every year.
However, even a very high rate of type-I supernovae can only partly explain why large amounts of iron were produced at such an early phase in the development of the universe. It is also likely that more time is needed to produce the iron, i.e., a larger age of the universe at the redshift of APM 08279+5255. In this way, we find independent evidence for the existence of the recently discovered "cosmological constant", a kind of "dark energy" which still pushes the universe apart.
The observations of APM 08279+5255 carried out with XMM-Newton provide important new information on nucleosynthesis and the chemical evolution of the early universe, on the new "unified models" for different types of quasar activity, and on the measurement of cosmological parameters like the cosmological constant. Presently, at these high redshifts we can only study very few, particularly luminous objects, like APM 08279+5255, using the XMM-Newton. In the future, however, scientists hope to use XEUS, the future large X-ray observatory of ESA, to routinely analyze X-rays from many faint distant objects, in order to answer the questions emerging from the present discovery. ●

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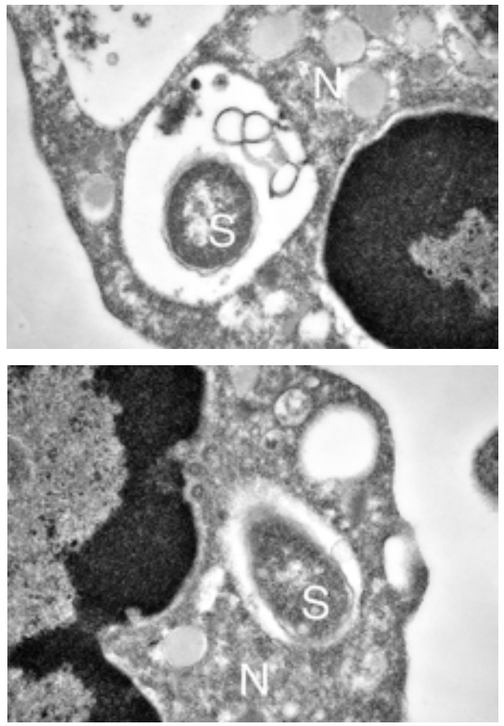

Photos: MPI for Infection Biology

Human neutrophils infected with *Shigella*. The bacteria are 10 times smaller than a neutrophil, so the pictures only show detail of the neutrophil where the *Shigella* are present. "S" indicates *Shigella* and "N" the neutrophil. In the top picture, the elastase is active and the *Shigella* is inside a vacuole. In contrast, the elastase in the lower picture is inactive and therefore the *Shigella* has "escaped" the vacuole.

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Infection Biology

A New Weapon for Combating Dangerous Bacteria

A constant battle is being fought inside our bodies between disease-causing bacteria and the immune system. The first line of attack against these types of intruders are the white blood cells which capture and eliminate microorganisms. Prof. Arturo Zychlinsky, Director of the Max Planck Institute for Infection Biology, and Yvette Weinrauch, from the New York University School of Medicine, have now discovered how white blood cells are able to neutralize the bacterial defences that cause disease using the enzyme elastase (Nature, 2 May 2002). This discovery provides a new basis for the development of a new generation of antibiotics tailored to combat dangerous infectious diseases such as dysentery or typhoid.

In the human body, white blood cells (leukocytes) capture invading bacteria. The white blood cells imprison the bacteria in tightly fitting compartments or vacuoles surrounded by a membrane. Within these vacuoles, the cells release noxious substances that kill the bacteria. This is why most of the time our bodies can take care of small wounds or mild infections. Sometimes, however, we get infected with bacteria equipped with highly evolved defences that target white blood cells, the very cells that are supposed to defend us. A case in point is dysentery, a serious, bloody form of diarrhoea. It is caused by bacteria called *Shigella*. This is a devastating disease that claims millions of lives every year, especially among children in the developing world.
Shigella uses disease-causing proteins to attack human cells. *Shigella* uses these virulent proteins to invade any cell in the body and then to escape from the cellular prison – the vacuole. During their investigation of this mechanism, Weinrauch and Zychlinsky recognized a contradiction: if *Shigella* escapes the vacuolar prison, the infection should always be lethal. Yet, fortunately, most people recover after suffering an acute infection.
Weinrauch and Zychlinsky reasoned that the answer could be found in a specialized white blood cell – the neutrophil. Neutrophils were prime suspects for two reasons. Firstly, they are very abundant in the response to *Shigella* infections, and secondly, they are equipped with defence mechanisms that kill bacteria when confined to a vacuolar prison. In collaboration with Jerry Weiss, now at Iowa University, the investigators found that surprisingly, neutrophils, unlike any other type of cells, can enclose *Shigella* within the vacuoles. The *Shigella* remains trapped within the vacuoles and cannot escape.
Investigations into the reason why *Shigella* is enclosed within neutrophil vacuoles led Weinrauch and Zychlinsky to discover how the neutrophil can neutralize not only *Shigella* but also other disease-causing bacteria. Neutrophils produce an enzyme, elastase, which can recognise and destroy *Shigella's* escape proteins with amazing efficiency before they leave the vacuole. This also includes the proteins that the *Shigella* need to escape from the vacuole. In this manner, neutrophils retain the *Shigella* long enough to mobilize other defences that can destroy the bacteria. Indeed, neutrophils with inactivated elastase cannot contain *Shigella* in a vacuole, and the bacteria remains virulent, as shown in collaboration with Steve Shapiro of Harvard Medical School. Interestingly, elastase destroys virulent proteins not only in *Shigella*, but also in Salmonella, the cause of typhoid fever, and *Yersinia*, the cause of bubonic plague.
Arturo Zychlinsky recognises important applications for his research findings: "We have still not answered a whole series of questions, such as: how is the elastase delivered at the right time? How can it distinguish between disease-causing and normal proteins within bacteria? Nevertheless, our new discovery will now allow the development of a new generation of antibiotics that rely on neutralizing the virulent proteins of disease-causing bacteria rather than indiscriminately killing microorganisms." ●

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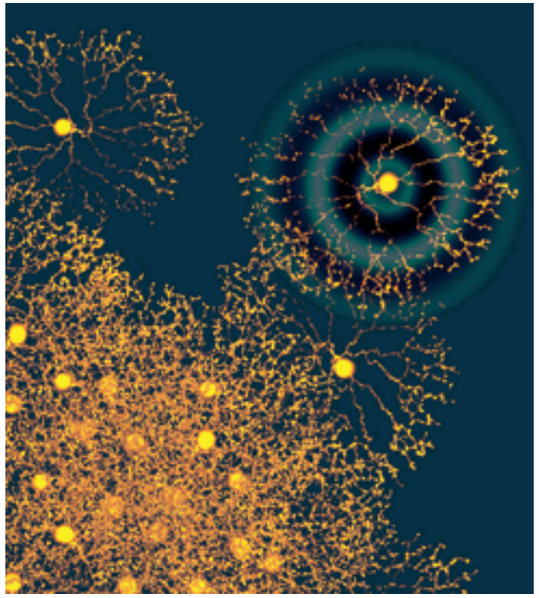
NEUROBIOLOGY

Watching the Retina Perform Neural Computations

The retina is a thin sheet of nervous tissue in the back of the eye that acts as a type of "projection screen". Not only does it work as a detector, but also as a highly specialized image processor: the retina analyzes visual scenes and extracts temporal, spatial and chromatic information, which is then relayed via the optic nerve to the higher visual centres of the brain. In the past, very little has been known about the extent of local signal processing in the dendrites – finely branched extensions – of the retinal nerve cells (neurons). Scientists at the Max Planck Institute for Medical Research in Heidelberg, along with the University of Washington in Seattle, have now succeeded in using a new imaging technique to produce high-resolution images of the living, light-sensitive retina and to investigate its information processing mechanisms. Using this "multi-photon laser scanning microscopy", the researchers discovered that areas within the same nerve cell can, to a large extent, act independently from each other to different directions of movement – and that they therefore possess several directional detectors (NATURE, Advanced Online Publication, 4 August 2002).

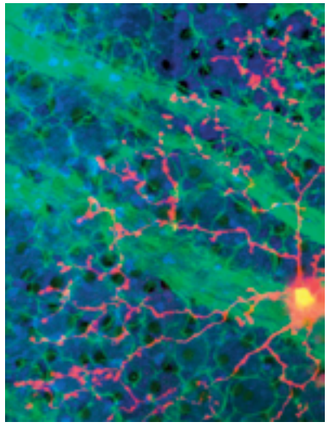
With their experiment, the Max Planck researchers Thomas Euler and Winfried Denk, in collaboration with Peter B. Detwiler from the University of Washington in Seattle, have progressed one step further towards solving a classic problem of retina research. As early on as 1964, scientists from Cambridge University discovered neurons in the retina which only respond when a visual image in the eye moves in a specific direction. At that time no one expected that it would take another 40 years to identify the nerve cells responsible for carrying out the neuronal computations necessary for this process. The cells identified by the Cambridge researchers belong to the output cells of the retina and are described as "direction-selective ganglion cells": they respond strongly when a stimulus moves in a specific direction, but show practically no response when the same stimulus moves at the same speed in the opposite direction. This means that the brain already receives information from this cell population on where the object in the field of vision is moving to.
The researchers therefore asked the following question: how and where does the retina carry out the computations necessary for this process to take place? Are the ganglion cells themselves responsible for calculating a direction signal from direction specific inputs, or do the ganglion cells receive direction-specific signals from other neurons?
In their study now published in NATURE the Heidelberg researchers concentrated on a specific type of retinal nerve cells which are also called "starburst" amacrine cells due to their characteristic shape. This cell type, which transmits signals to the direction-selective ganglion cells, had long been the leading candidate for carrying out the directional calculations. The stimulus responses electrically recorded at the cell body show no direction selectivity whatsoever; this means: the cells are not electrically direction-selective. However, in general, amacrine cells have no defined output process or "axon"; they use



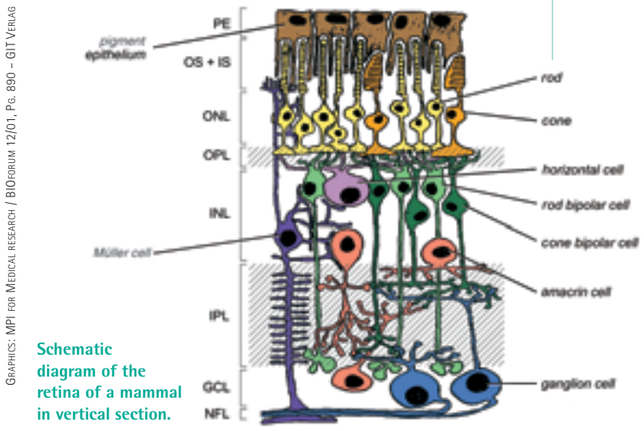
Multi-photon microscopical images of "starburst" cells: the picture on the right shows tissue, the one on the left shows a photo sequence of one of the light stimuli used.

PHOTOS: MPI FOR MEDICAL RESEARCH

their dendrite extensions to both receive and transmit synaptic signals. The dendrites process the input signals and in turn relay the resulting signal to, amongst other neurons, the direction-selective ganglion cells. The dendrites of nerve cells are often so thin that it is practically impossible to use microelectrodes to monitor their activity. In order to measure the biochemical signals – which are produced by calcium and serve as the means of communication between nerve cells – in the dendrites, the Max Planck researchers therefore used a new optical method: multi-photon microscopy based on a pulsed infrared laser. Although this light is a million times more intense that direct sunlight, it is not visible to the human eye as it is not absorbed by the light-sensitive visual pigments in the photoreceptors of the retina. The visible light normally required for stimulating the indicator dyes would blind the exceptionally sensitive retina within seconds. The extremely short but highly intense light pulses from the infrared laser

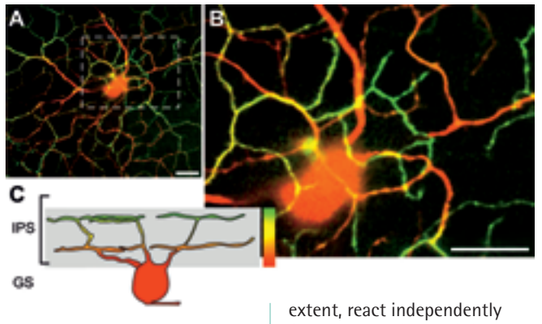


make the indicator dye molecules – previously injected into the cells under investigation – fluoresce in a non-linear process of multi-photon absorption. In contrast to conventional confocal microscopy where each indicator dye molecule is excited with one short-wave, high-energy photon, multi-photon microscopy uses two long-wave, low-energy photons.
This long wavelength (infrared) provides practically no stimulation to the photoreceptors and the light sensitivity of the retina is thereby conserved. In this way, it is now possible for the very first time to stimulate the retina using light patterns and to perform optical recordings of its neuronal responses simultaneously.
This technique enabled the researchers to measure fluctuations in the calcium ion concentration in the output contacts (synapses) of the "starburst" amacrine cells with greater temporal and spatial resolution. At the same time, they discovered that different areas within one and the same "starburst" cell could, to a large extent, react independently from one another and thereby have an individual preference for different movement directions. The dendritic output signals are consequently direction-selective with individual dendrites showing a preference for movement that is directed away from the cell soma, i.e. centrifugal image motion. This also explains why the electrical signal in the cell body appears to be unspecific – the signals from the dendrites are averaged out. In other words: each "starburst" amacrine cell contains several direction detectors.



Schematic diagram of the retina of a mammal in vertical section.

GRAPHICS: MPI FOR MEDICAL RESEARCH / BIOFORUM 12/01, PG. 890 – GIT VERLAG



Reconstruction of a direction-selective ganglion cell (A; detailed image shown in B). The dendrites of the cell branch out onto two levels and were subsequently highlighted in two different colours. The diagram (C) shows a side view of the position of the cell and its dendrites and explains the colour coding. The white line shown in A and B is equivalent to 20 thousands of a millimetre.

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NEUROBIOLOGY

Genetic Defect Makes Mice Hit the Bottle

Clinical and epidemiological investigations have shown that humans who are sensitive to stress can become addicts if exposed to increased levels of stress. In their article published in SCIENCE (2 May 2002), scientists led by Prof. Florian Holsboer from the Max Planck Institute for Psychiatry in Munich confirm the results of these investigations and thereby point out new possibilities of preventing the development of stress-induced alcohol abuse through the use of medicines which target either the stress hormone or glutamate system. This could provide therapeutic help to humans with a genetically-induced alteration of the stress regulation system.

Stress increases alcohol consumption in mice with the CRH receptor type 1 switched off.

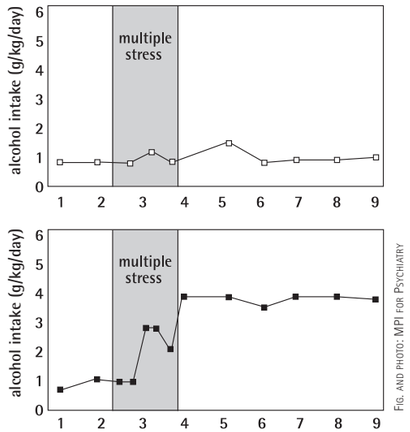

FIG. AND PHOTO: MPI FOR PSYCHIATRY

The development of alcoholism can be attributed to both genetic causes and environmental influences. One particularly important environmental factor is stress – and not just with regard to alcohol abuse, but also in the case of other psychiatric disorders, such as anxiety disorders, depression, and illnesses caused by posttraumatic stress. The genetic disposition of the individual affected plays an important role in the development and extent of alcoholism. It is interesting to observe that this relationship also applies to laboratory mice.

With the aid of molecular-biological techniques, scientists at the Max Planck Institute for Psychiatry have succeeded in creating a new mouse model in which the mice have been genetically altered to lack a key component in their stress response system. If the organism is exposed to a stressful situation, it will increase its release of a protein molecule, the corticotropin-releasing hormone CRH. This molecule not only controls the hormonal stress response but also coordinates a whole series of behavioural responses which are suitable for overcoming the stress situation. CRH bonds in various regions of the brain which are relevant to emotional behaviour such as anxiety. The "corticotropin-releasing hormone receptor type 1" is one of the receptors which pick up the hormonal signal. If this receptor is switched off in a knockout mouse, then the central stress reaction is disturbed. The knockout mouse developed by the scientists in Munich has a genetic defect in the precise gene which carries the building instructions for the CRH receptor type 1.

The researchers at the Max Planck Institute for Psychiatry in Munich (Inge Sillaber, Gerhard Rammes, Stephan Zimmermann, Beatrice Mahal, Walter Zieglgänsberger, Wolfgang Wurst, Florian Holsboer, and Rainer Spanagel) offered alcohol to their knockout mice. In their investigations, the mice that had been genetically altered to lack a key component in their stress response system initially consumed the same quantity of alcohol as the genetically-intact animals in the control group. However, when the knockout mice were repeatedly exposed to stressful situations for short periods of time, they reacted differently from the control group over a period of five months by progressively increasing their alcohol intake. An intact central stress system is clearly necessary to reduce or prevent the risk of alcoholism which occurs following repeated exposure to stress.

Parallel investigations showed that increased levels of another receptor in the brain (NR2B), with which glutamate bonds, are present in specific regions of the brain in the knockout mice developed by the Max Planck scientists: this receptor is sensitive to alcohol. For this reason, the scientists suspect that the increase in the glutamate receptor in the knockout mice contributes to the stress-induced increase in alcohol consumption. ●

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MICROSTRUCTURE PHYSICS

Nanotubes Made from Teflon

In the past, nanotubes with diameters measuring anything from just a few nanometres right through to several hundred nanometres could only be manufactured out of a limited range of materials, such as carbon. Interesting materials with beneficial characteristics – for example, polytetrafluor ethylene (Teflon), light-emitting polymers, copolymers, or material compounds with a defined composition – were unsuitable for production. Chemists and physicists from the Max Plank Institute of Microstructure Physics in Halle and the Institute of Physical Chemistry at the Phillips University Marburg have now succeeded in developing a universally applicable procedure, which enables nanotubes to be formed from a variety of materials and compounds (SCIENCE, 14 June 2002).

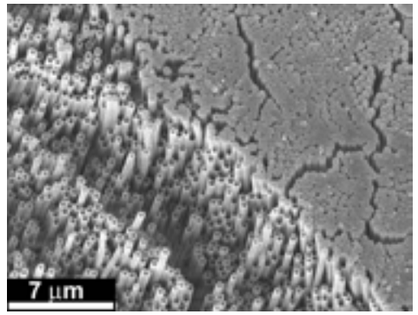
To produce the nanotubes, the researchers used small plates made from nanostructured silicon or aluminium oxide which consist of highly ordered nanopore arrays. These pores were created by means of self-organisation, lithography, or by a combination of both methods. If liquid polymers or solutions containing polymers are brought into contact with these porous structures, a thin film with a thickness of around 20 nanometres (billionths of a metre) will form on the pore walls. When the solvent is cooled or evaporated, this film will solidify and form nanotubes. The shape and size is determined by the pores and this means that the pore structures used act as a type of "template". If the material from which the pore structure is made is selectively removed, then we are left with the nanotubes. According to the particular type of pore structure used, the tubes are all the same size. In this way, it is even possible to produce highly ordered arrangements of parallel nanotubes.

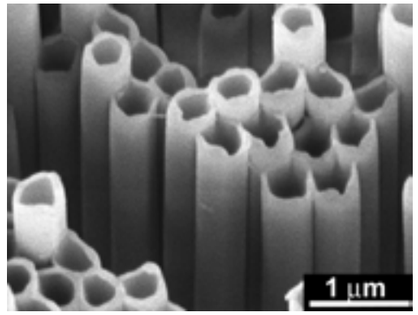
For the very first time, the scientists created nanotubes from polytetrafluor ethylene (Teflon) – a polymer that had previously remained difficult to structure in the nanometre range, due to its particular characteristics, but which offered great application potential. In principle, it is now possible to produce nanotubes out of practically any type of polymer that can be processed in the form of a melt or out of a solution including, for example, polystyrene or polymethyl methacrylate.

One major advantage of this new method: the polymers used can be added to other materials and mixed to produce composite nanotubes. The walls of these composite nanotubes can consist, for example, of a mixture of polystyrene and palladium, a metal which is of great importance in the fields of catalysis, sensor technology, and fuel cells.

The new method opens up a whole variety of application possibilities. It could provide porous materials with special functions through the use of inner coatings. These materials could then be used as tunable photonic crystals in integrated optics or as special carrier plates (arrays) with millions of microcavities in combinational chemistry.

On grounds of their biocompatibility, pore structures coated with polymers using this method could one day be used to analyze blood brain barriers which block the transfer of most substances, including pharmaceutical agents, into the brain. Within the framework of a nanobiotechnology project supported by the German Federal Ministry of Research, led by the University of Münster, the scientists are placing living cells on polymer coated pore structures and are investigating the transport of agents through these cells into the pores located underneath. ●





Top: nanotube arrays made from polystyrene shown in various magnifications. The tubes have a diameter of around 400 nanometres. Below: individual polystyrene nanotubes.

PHOTOS: MPI FOR MICROSTRUCTURE PHYSICS

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RESEARCH *in Brief*

METEOROLOGY

The Clouds Have Lifted after Glasnost

Since the end of the Cold War the temperatures in Central Europe have risen at an even greater rate. This statement summarizes the findings that have recently been obtained by Prof. Hartmut Graßl, Director of the Max Planck Institute for Meteorology in Hamburg, and Dr. Olaf Krüger from the Meteorological Institute of the University of Hamburg, from a series of satellite measurements conducted over a period of almost twenty years and published in the journal GEOPHYSICAL RESEARCH LETTERS (October 2002).

The scientists themselves speak of a "Gorbachev effect" – defined by Graßl as follows: "After 1989 the clouds over Central Europe, observed from outer space, have become darker. This means that they no longer reflect as much sunlight back into outer space as before, but instead allow more radiation to be transmitted through to the earth. And for this reason it is now becoming warmer under the clouds." This change in the reflecting ability of the clouds is a result of the wave of environmental-political clean-up work that swept through the former German Democratic Republic, the Czech Republic, and Poland following the lifting of the Iron Curtain: in this region, formerly known as the notorious "black triangle", countless industrial plants and power stations, which had previously pumped out vast quantities of pollutants into the lower atmosphere, were closed down or redeveloped following this turning point. After more than a decade, this enormous "clean-up campaign" has turned out to be a type of large-scale experiment involving a particular problem that affects climate research – one that up until now has remained purely theoretical but nevertheless highly topical – the problem of the so-called indirect aerosol effect. Aerosols are fine droplets or solid particles with diameters of between one hundredth and one ten thousandth of a millimetre which are suspended in the air and can remain and disperse in the atmosphere for several hours or for anything up to several weeks. These floating particles influence the radiation levels and therefore the climate near the ground in two ways: firstly, they have a direct influence by dispersing or absorbing not only light but also heat radiation, and secondly, they exert an indirect influence by affecting the formation of clouds, influencing their optical characteristics and life span. The second effect, the indirect aerosol effect, is primarily responsible for making the tiny floating particles become an important factor in the climate system – but also at the same time a factor of uncertainty for climate models, as the influence of aerosols on clouds could previously only be estimated using theoretical calculations. This is due to the fact that two competing mechanisms are involved in the indirect aerosol effect. On the one hand, aerosols above a certain size act as condensation nuclei for water vapour and thereby determine both the number and size of the water droplets formed. The more aerosols a cloud contains, the larger the number of droplets formed in it and the smaller their size – a process that in turn is increased by its reflecting ability: the cloud then reflects more sunlight back into outer space and the ground becomes cooler. On the other hand, aerosols – particularly those formed from dark soot or ash particles – also act as radiation traps. They absorb light and in this way heat up the clouds. This can take place to such an extent that soot saturated clouds are literally evaporated away and disperse.

This "game of doubles" is hard to calculate, a task that is made all the more difficult by the fact that the aerosol mix of the lower atmosphere is fed from numerous sources: both from natural sources, such as salt spray from the oceans, from the burning of vegetation or from volcanoes, and also from anthropogenic emissions from industry, domestic heating, or traffic. Accordingly, the type and concentration of the aerosols are subject to both spatial and temporal fluctuations within wide ranges – and for this reason it has so far only been possible to estimate the indirect aerosol effect in climate models on a global and general basis: the current assumption made by scientists is that on the whole it has a cooling effect and reduces the radiation flux into the lower atmosphere on a global basis by up to two watts per square metre. It would therefore counteract and significantly slow down the additional anthropogenic greenhouse effect – which is currently estimated to be responsible for increasing radiation flux by 2.5 to 3 watts per square metres.

Industrial emissions influence the aerosol content of the atmosphere and therefore the radiation flux on the ground.

Clouds reduce the radiation flux in the lower atmosphere and play an important role in climatic events.

PHOTOS: OKAPIA, MUNICH

However, the researchers are not entirely certain about the extent of this slowing-down effect. For this reason, climate models relating to the increase in the temperature on the Earth in the coming decades still leave plenty of scope for interpretation. This is how Graßl and Krüger hit upon the idea of investigating the effects of the turning point on the clouds above Central Europe using a before-and-after comparison – in the hope of obtaining, for the very first time, concrete data – i.e. measurement-based data – on the influence of the indirect aerosol effects on the radiation levels on the ground. This hope was based on the fact that, along with the massive industrial emissions that were being churned out into the skies by the "black triangle" up until 1989, significant quantities of so-called precursor gases also found their way into the atmosphere – sulphur dioxide and nitrogen oxides from which aerosols are formed in the air by means of chemical reactions. In addition to this, a significant emission of soot and ash particles containing carbon created a further abundant source of aerosols. The quantity of these "aerosol seeds" fell drastically following the political turning point within a space of only a few years – and this included above all the emission of sulphur dioxide, the most significant precursor gas in terms of quantity: between 1988 and 1998 this was reduced by almost half with regard to the whole of Europe.

Graßl and Krüger suspected that this rapid reduction in anthropogenic aerosol sources might also have had an effect on the clouds above Central Europe as a result of the indirect aerosol effect. In order to determine this, the climate researchers from Hamburg enlisted the help of several American satellites which had recorded the reflecting ability of the clouds, including those above Europe, over a period of several years using sensitive spectrometers. This data was supplied by two long-term measurement series – one covering the period from 1985 to 1989 and the other 1996 to 1999. These two measurement series were then analyzed and compared in terms of the reflecting ability of the clouds – divided in each case according to winter and summer periods.

The view from outer space, supplied by the satellites, provided the very first differentiated insights into the complicated interplay between aerosols and clouds. It revealed a highly variable pattern with regard to the "radiation strength" measured from above, both in spatial and temporal terms. Emission centres, such as conurbations or industrial regions during the winter months were generally characterized by a clear reduction in reflecting ability – a consequence of the increased soot and ash particles which absorbed the incoming light in clouds. In contrast to this, during the summer months emission centres stood out by an increased radiation reflection, caused by the predominant "droplet effect", i.e. by the formation of ever-increasing numbers of smaller water droplets within the clouds. These and other detailed findings provided fundamental evidence, for the very first time, that the indirect aerosol effect really does have an influence on the clouds over Central Europe. Furthermore, a before-and-after comparison of both measurement series revealed that Graßl and Krüger were indeed correct in terms of the "Gorbachev effect". On average, the reflecting ability of the clouds over Central Europe in the years after 1989 has dropped by 2.8 percent and the radiation flux onto this region has strengthened accordingly by about 1.5 watts per square metre. That is to say, the anthropogenic greenhouse effect which was previously masked due to aerosols from the industrial emissions of the "black triangle", is now making its mark on Central Europe with increasing strength.

Or in other words: the change in the political change has paved the way for the anthropogenic climate change.

This discovery came as no surprise to Graßl. As a specialist in aerosols he had already alluded some twenty years ago to the possibility that measures taken to clean up the air could have this kind of "perverse" effect. At that time, he was confronted with considerable reservation and even criticism. Now that his predictions have been shown to be correct, he makes the following comment: "Only those who jointly tackle all the causes of climate changes, that is to say achieve emission reductions, in the case of long-life carbon dioxide and short-life gases such as sulphur dioxide and soot, will slow down the rapid climate change without further distortion. ●

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