# Bacteria synthesize brain hormone

Molecular geneticists at the University of California in San Francisco have just completed work that not only eclipses all previous gene-engineering research, but may mark the beginning of a new era in the biological sciences as well.

Scientists from three West Coast institutions (led by Herbert Boyer of UCSF) have succeeded in manipulating a colony of bacteria to produce a human brain hormone, thus delivering on a major promise of researchers who discovered a way to splice genes together more than five years ago. The successful study was announced by Philip Handler, president of the National Academy of Sciences, who testified before a Senate subcommittee that the experiment "was a scientific triumph of the first order."

Research teams led by Boyer, Arthur Riggs of the City of Hope Medical Center near Los Angeles and Wylie Vale of the Salk Institute in San Diego produced 5 mg of somatastatin, a mammalian protein neurohormone. They did it by inserting an engineered gene into about 100 mg of *Eschericia coli* suspended in 2 gallons of culture medium. The bacteria heeded the new "work orders" and, in Handler's phrase, like bustling factories "merrily engaged" in producing the hormone.

That the researchers chose to produce somatastatin was incidental, Handler said. Even though it took Roger C. L. Guillemin of the Salk Institute 500,000 sheep brains to accumulate the 5 mg of somatastatin he needed to decipher the hormone's structure—for which he shared the Nobel Prize in medicine this year (SN: 10/22/77, p. 260)—the hormone can now be produced in organic laboratories relatively cheaply. (Somatastatin, secreted by the hypothalamus in trace amounts, inhibits the pituitary gland's release of hormones that regulate body growth and glucagon and insulin production. It may be useful in the future treatment of diabetes, pancreatitis and acromegaly, a disease of abnormal bone growth.)

Nor was this the first time researchers have been able to introduce foreign genes into bacteria. Another team of UCSF researchers accomplished that earlier in the year by inserting a rat gene that codes for insulin production into *E. coli* (SN: 5/28/77, p. 340).

The insulin gene did not trigger the production of rat insulin by the bacteria, but the somatastatin researchers did induce the *E. coli* to ignore its own functions and, like a surrogate mother, mistakenly cultivate a metabolic process normally found only in mammals.

How the geneticists "tricked" the *E. coli* is not precisely known, but other workers in the field say it was a striking conceptual departure from earlier attempts at UCSF, which inserted natural genes into *E. coli*. (The rat gene researchers used messenger RNA, which carries genetic information from the cell nucleus to the protein-making machinery in the cytoplasm, as a "negative" to make a "positive" copy of the original gene.) Instead, Boyer and his co-workers constructed a gene from scratch, successively adding nucleotides like beads on a string. This artificial chain of nucleotides coded for the amino acid methionine as well as the amino acids which make up somatastatin.

According to a colleague from another department at UCSF, the researchers then linked a natural bacterial chain, the beta-galactosidase gene, and its control sequence to the artificial gene. Presumably, this natural gene sequence was added to "prime" the bacteria—while expressing the beta-galactosidase gene it would also express the artificial gene attached to it.

The next steps were relatively routine. This chain of "recombined" DNA, the nucleotides coding for methionine plus somatastatin plus beta-galactosidase, were spliced into either a virus or a bacterial plasmid, which was then introduced into some of the bacteria in the colony. After waiting for the bacteria to follow the new genetic blueprints, producing a large peptide chain containing both somatastatin and methionine, the researchers liberated the brain hormone with a chemical process that cleaved it from the methionine.

The researchers, however, refuse to confirm or deny this reported experimental design. According to a spokesman at UCSF, Boyer and his team are adhering to the traditional policy of withholding comment on a specific experiment until its methods and results have been "refereed" by a scientific journal, and then published. "Handler," the spokesman said, "must have heard of the research through the scientific grapevine. It was certainly not our idea—in fact, it caught us by surprise."

Handler's announcement probably also caught Genentech by surprise. Genentech is a California company Boyer organized two years ago to construct synthetic gene sequences that would be used to produce valuable medicinal drugs, such as insulin and possibly somatastatin. In testimony before the Senate subcommittee on science, technology and space, Boyer told Chairman Adlai E. Stevenson (Dem-Ill.) Genentech had paid for the somatastatin research through a contract with UCSF. UCSF, which is applying for federal patents protecting Boyer's new techniques is bound by the contract to award licensure to Genentech, would pay UCSF royalties on profits earned by such patents. A source familiar with the UCSF work said Boyer, worried that public discussion of the new techniques would prejudice chances for patent approval, had advised his fellow researchers to say nothing more about the experiment.

Handler may have preempted any later announcement of the experiment by Boyer in order to bolster his and other scientists' testimony before the same subcommittee (on Nov. 3) that not only was recombinant DNA research safe, but that it also (in the words of Paul Berg of Stanford University) "puts us at the threshold of new forms of medicine, industry and agriculture." □

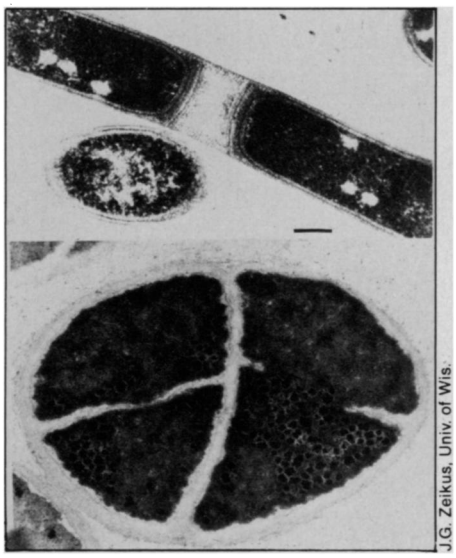
# Methanogens: A third branch of life

The tree of evolution may need to be remodeled to reflect recent research results on the genealogy of microorganisms. A collection of twigs sometimes scattered on one side of the tree's major bifurcation may have to be regrouped into a third, and new, division of the trunk. It is suggested that members of this newly-proposed line of evolution changed little over the millenia and therefore resemble ancestral life forms dating back 3 to 4 billion years.

The newly-proposed evolutionary line contains all bacteria that produce methane, including *Methanobacteria, Methanospirillia* and *Methanosarcina*. But according to the researchers these groups should no longer be called bacteria. Carl R. Woese, leader of the team that is proposing the phylogenetic change, suggests they be renamed "archaebacteria" in deference to their proposed age.

Although they may have once dominated, methane-producing bacteria today fill only scattered, oxygen-free niches, such as hot springs in Yellowstone Park and the mud under the San Francisco Bay. They thrive on hydrogen and carbon dioxide and create methane gas ($CH_4$) as waste. Thus these microorganisms are called methanogens (methane producers).

Woese and his colleageues at the University of Illinois have been measuring the genealogies of organisms. They use a quantitative technique that Woese compares to the method one scholar used for dating cookbooks. The scholar determined which book was copied from which by tracing misspellings that crept in and were then included in later editions. Woese and co-workers charted the species differences among cellular macromolecules. The variations result



J.G. Zeikus, Univ. of Wis.

*Candidates for a third evolutionary line: One species (top) forms a chain of two organisms (also seen in cross section); another species about to undergo division.*

from changes that occur once and are then copied into all descendants.

The "page" which Woese chose to examine in the volume of macromolecules that characterize an organism is the RNA found in ribosomes. Because ribosomes are an essential part of the machinery for converting genetic information into protein, they are common to all organisms from methanogens to humans. "Every self-replicating system has it," Woese says. He explains that the sequence of nucleotide "letters" in ribosomal RNA seems to be tightly controlled. Misspellings crop up less frequently than in genes, for example.

In tackling the phylogeny of bacteria, Woese asked other researchers to suggest groups of microorganisms for his study. He says, "I ask them, 'What's a peculiar bacteria by the criteria you're using?'" Ralph S. Wolfe, a microbiologist at the University of Illinois, suggested the methane producers.

Woese and colleagues have now compared one type of ribosomal RNA isolated from 12 different methane-producing bacteria and 60 other bacteria (which Woese suggests calling eubacteria or true bacteria). The sequences of those groups bear little resemblance, the researchers conclude. Some "words" that occur among the methane producers never occur in other bacteria. Other sequences of nucleotides found in almost all bacteria were never detected in methanogens.

But is is comparisons with the higher life forms that lead the researchers to propose that the methane producers are not just very distant bacterial relatives. "They are just as close genealogically to higher forms as to bacteria," Woese says. So far the researchers have done only a broad screening of higher life forms. They have examined one animal, one plant, one yeast and one slime mold, Woese told SCIENCE NEWS.

Although the genealogy is the clearest indication that the methane-producing bacteria are a distinct evolutionary group, other differences separate them from typical bacteria. Wolfe has found at least three coenzymes (the nonprotein portion of enzymes) that are unique to methanogens. Some other common components have not been detected in those microorganisms. Almost all cell walls contain peptidoglycan, but the walls of the methane-producing bacteria do not. Finally, bacteria and higher organisms have the exact same sequence of bases in one region of transfer RNA, the cell component that carries specific amino acids to a forming protein chain. Methane-producing bacteria are the first major group of organisms in which that unique nucleotide sequence has not been found. All these differences contribute to the researchers' belief that methanogens form a systematic group distinct from other bacteria.

The idea that methane-producing bacteria provide a glimpse of ancestral life has two supporting arguments, Woese says. The first is the genealogic evidence that branches within the methanogen group split long ago—at least as far back as when the blue-green algae split from bacteria. Yet the biochemistry of all the methane-producing organisms is similar, as if those organisms simply have evolved slowly. "There is a constant kind of biochemistry across a deep, ancient division," Woese says. From this he infers that earlier life forms, before methanogens, bacteria and plants and animals split, relied on similar chemical reactions.

Conditions of the primitive earth provide the other basis of support for Woese's suggestion that methane-producing bacteria are the common ancestor. "Their requirements for growth are like the primitive atmosphere," he points out. "They can't stand oxygen and they live off $CO_2$."

The researchers suggest that the methanogens may even have played a pivotal role in the earth's physical evolution. Methane-producing organisms might have digested much of the cloud of carbon dioxide that once enveloped the planet, making possible evolution of higher life forms, Woese speculates.

Reports of the work by Woese, Wolfe, George E. Fox, Linda J. Magrum and William E. Balch will appear in the October and November PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

There are no fossil records to corroborate the proposed genealogy; methanogens may have been forming before there were rocks on earth. Therefore, scientists have only their analyses of the record within cells' genes and protein. As they examine more genealogies, Woese says, biologists may uncover yet other distinct evolutionary lines of life. □

# Planetoid between Saturn and Uranus

It's almost certainly not a comet. It would be misleading to call it an asteroid. Nor is it a moon in orbit around a planet. But whatever it is, it's out there circling the sun between the orbits of Saturn and Uranus.

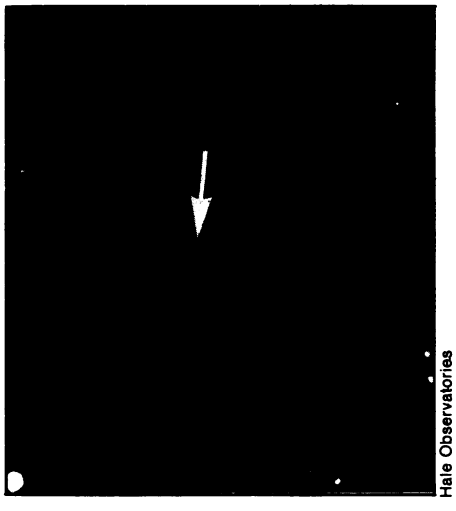
It was discovered by Hale Observatories astronomer Charles Kowal, who has been credited in the past with discoveries ranging from supernovae to the 13th and possible 14th moons of Jupiter. He first spotted the object in a photographic plate taken on Oct. 18 with the 48-inch Schmidt telescope on Palomar Mountain, then found it again in a plate from Oct. 19. Next it was located by University of Arizona astronomer Tom Gehrels, looking back at plates made on Oct. 11 and 12, after which California Institute of Technology graduate student Richard Green photographed it on Nov. 3 and 4.

The combined observations span a period of less than three weeks, making it difficult to be certain of the orbital elements (although at magnitude 18 or 19, says Kowal, the object is bright enough that it should be relatively easy to find in past plates). However, Kowal says, the object seems to take from 60 to 120 years to circle the sun, in a circular or slightly eccentric path with an inclination of 3 to 5 degrees. Its brightness implies that, if it has a surface like that of earth's moon, it is about 300 miles across, Kowal says; a darker surface like a carbonaceous chondrite would mean that it is larger, while an icy, more reflective surface would make it smaller. Photometric observations are likely to be made in the near future.

The object's image on the plates is too sharp, and its orbit too shallowly inclined, for it to be a comet. It should not be considered an asteroid, according to Kowal, since that implies a location between Mars and Jupiter and perhaps restricts the list of source mechanisms.

But what is one to call it? A planetoid? "That would be a nice name for it," says Kowal, "if only we could revive it." □

*"Object-Kowal"—but what is it really?*



Hale Observatories