SCIENCE NEWS®
A Science Service Publication
Vol. 114/September 16, 1978/No. 12

COVER: Liquid nitrogen freezers hold samples at a temperature where no biological or chemical deterioration can occur. Research into freezing and thawing provides techniques for indefinite preservation of animal and human sperm and of early embryos from six species. See p. 202. (Photo collage: National Institute on Aging/Dale Appleman)

Editorial and Business Offices
1719 N Street, N.W.
Washington, D.C. 20036

Subscription Department
231 West Center Street
Marion, Ohio 43302

Subscription rate: 1 yr., $12.50; 2 yrs., $22; 3 yrs., $30. (Add $2 a year for Canada and Mexico, $3 for all other countries.) Change of address: Four to six weeks' notice is required. Please state exactly how magazine is to be addressed. Include zip code.

Printed in U.S.A. Second class postage paid at Washington, D.C. Title registered as trademark U.S. and Canadian Patent Offices.

Published every Saturday by SCIENCE SERVICE, Inc. 1719 N St., N.W., Washington, D.C. 20036. (202-785-2255) TWX 710-822-9433 SCIEN NEWS. ISSN 0036-8423

Human Insulin: Seizing the Golden Plasmid

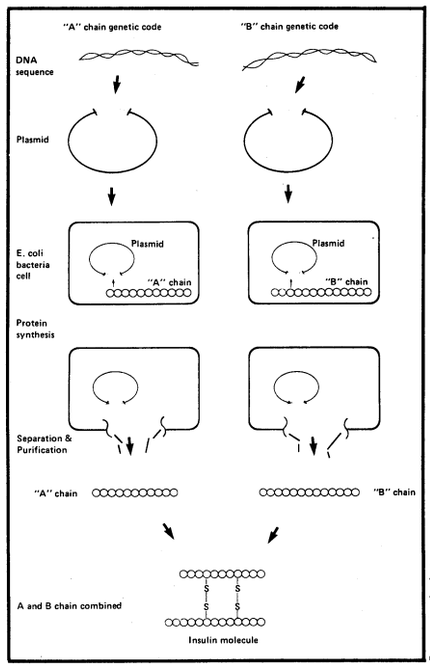
Human insulin has been produced at last by genetically engineered bacteria in a California laboratory — an achievement that catapults recombinant DNA technology into the major leagues of the drug industry. Immediately following the report of the research success, Eli Lilly and Co. announced an agreement with Genentech, Inc., a two-year-old research firm in San Francisco that sponsored and participated in the research. Lilly is the major supplier of animal insulin now taken daily by about a million diabetics.

The strategy that produced human insulin is the same as that used last year to make the simpler hormone somatostatin (SN: 11/12/77, p.310). Researchers chemically link together the nucleotides corresponding to the known amino acid sequence of the hormone. Because insulin is made of two protein chains, Roberto Crea, Tadaaki Hirose, Adam Kraszewski and Keiichi Itakura of the City of Hope National Medical Center near Los Angeles synthesized an artificial gene for each chain. The Genentech scientists then inserted the genes into plasmids, rings of natural bacterial DNA, that carried the genes inside bacteria. The plasmid, including the artificial gene, is reproduced as the bacteria multiply.

The researchers used the same trick as before to coax the bacteria into large-scale production of the desired hormone. The artificial gene was snapped into the plasmid among a group of genes that scientists know how to regulate. In the presence of lactose (milk sugar) the bacterium switches on those genes. The artificial gene for insulin, now linked to the gene beta-galactosidase, also is activated. In the recent work each bacterium was induced to produce 100,000 molecules of either chain A or chain B human insulin.

The A chain of human insulin, with its 21 amino acids, was purified from the bacteria with artificial A gene in their plasmids. The B chain of 30 amino acids was obtained from bacteria with an artificial B chain gene. The chains were purified and combined by Crea and Arthur Riggs of City of Hope and David Geoddel of Genentech. Ten to 40 percent of the material formed the two appropriate chemical bonds to become complete insulin. The simpler somatostatin, produced last year, has only one chain with 14 amino acids.

The strategy used by the California researchers differs from the procedure recently reported for producing rat insulin in bacteria (SN: 6/17/78, p.388). The Boston scientists did not chemically synthesize, link by link, the rat insulin gene they inserted in the plasmid, but copied it from the rat's natural gene. That approach might ultimately be advantageous for larger proteins, because chemical synthesis of long genes remains tedious.



Genentech, Inc.

Recipe for human insulin. Large-scale production expected in two to five years.

Genentech spokesmen would not speculate on when human insulin might reach the pharmaceutical market, but Rachmiel Levine of City of Hope predicted large-scale production in two to five years. First the developers have to scale up their laboratory procedures for commercial use. Then they must perform animal and clinical safety tests. Genentech says the researchers have followed the National Institutes of Health guidelines voluntarily during the laboratory development and plan to work closely with NIH in the future.

Genentech has set up a pilot manufacturing plant to find techniques suitable for commercialization for growing bacteria and extracting the hormone chains. *Escherichia coli,* in the bacterium's natural genetic state, is already used to produce L-asparaginase for medical use and other microorganisms make antibiotics; so there are precedents for bacterial drug manufacture. Under the agreement with Lilly, the companies will jointly develop the technique. Lilly will pay for the right to produce and market the insulin, but Genentech will remain owner of the technology. Genentech has already filed a number of patents for the techniques employed in human insulin production, techniques that the firm expects to be applicable to other products.

"The development of human insulin demonstrates the viability of using recombinant DNA technology to produce

products with practical application," says Robert Swanson, president of Genentech. Swanson says the company has another contract with an international pharmaceutical company to attempt development of other hormone products, vaccines and anti-viral products. Genentech is also considering using recombinant DNA to produce catalytic enzymes for basic industrial processes. But right now they claim bacterial synthesis of human insulin as possibly the most significant advance in diabetes treatment since the use of animal insulin began in 1920. □

Upsilon prime: 10 GeV excited state

To say that a physical system is in an excited state is to say that it has more energy than it had before it got excited. Excited states throw a great deal of light on the physics of matter (quite literally — from a lamp, for example). From the way in which excited states come and go and from what they absorb and emit in doing so (their spectroscopy) comes a knowledge of the structure and behavior of physical systems, be they molecules, atoms, nuclei or subnuclear particles.

The heaviest subnuclear particle ever found, the upsilon, is now on the way to becoming the heaviest family or spectroscopic hierarchy of particles. The Deutsches Elektronen-Synchrotron laboratory (DESY) in Hamburg announces that its DORIS storage ring has found an excited state for the upsilon, which is being called upsilon-prime.

The original, simple, upsilon was discovered last year at the Fermi National Accelerator Laboratory in Batavia, Ill. Its existence was confirmed by DORIS in May of this year. Its mass is 9.46 billion electron-volts (9.46 GeV), about ten times the mass of the proton or slightly more than the mass of a beryllium atom. The upsilon-prime has a mass of 10.02 GeV by the best reduction of data to date. The mass difference, which is equivalent to energy, represents the excitation.

Upsilon-prime would thus be the heaviest subatomic particle yet discovered if you accept excited states as new particles, which most particle physicists tend to do. (In atomic physics an excited state of an atom is just the same atom with a little more energy; there are reasons of history and principle for the difference.) So now the answer to the question how massive a particle can be has passed the 10 GeV barrier.

Probably no one expected that it wouldn't, but making one that heavy is nevertheless an achievement. The real importance of the existence of an excited state for the upsilon, as the DESY announcement stresses, is that it goes to prove that the upsilon must be made up of at least two subunits, which, as in atoms, are able to move with respect to each other. If not they couldn't take up the energy that makes an excited state. The upsilon-prime is considered to be the same basic structure plus the extra energy.

Theorists have a prescription concerning what those two constituents should be: They should be a quark possessing the quality designated "b", which is glossed by the DESY announcement as "beauty" but in the United States is usually called "bottom," and the antiquark possessing antibeauty.

Quarks are the unobserved subunits that theory now believes make up the structure of the observed particles. Each quark embodies a particular physical property that is important in the nature and behavior of particles. The names of the qualities can be whimsical, like beauty, and are as arbitrary as any terms in physics. When we say something is electric, for example, we no longer mean it is covered with amber.

Upsilon was the first particle discovered that is believed to contain beauty (or bottom). Four other varieties of quark are believed present in known particles. The sixth remains to be found. Now that there is an upsilon-prime (and there may yet be other members of the family) studies of the spectroscopy of beauty may begin. The difference in mass between the upsilon and the upsilon-prime gives some information about the force between beauty and antibeauty; a more numerous group would help even more. If upsilon-prime can be observed to decay into upsilon, then whatever it emits will also give important information about how beauty is and does and could be a new particle or particles itself. □

NCI Laetrile review inconclusive

Laetrile has not been found effective in test-tube and animal experiments (SN: 8/6/77, p. 92), but public pressure for its legalization by the U.S. Food and Drug Administration remains high. Seventeen states currently allow its use in spite of FDA prohibition of its interstate commerce, and 70,000 Americans are currently estimated to be using Laetrile. Consequently, the National Cancer Institute, in spite of its usual stance of only clinically testing drugs already shown effective in animals, is considering running a clinical trial to see whether or not Laetrile is effective against human cancer.

Before the NCI undertakes such an unorthodox mission, though, it wants a better idea of what evidence already exists for Laetrile's clinical effectiveness. Last January, the NCI requested that the nation's Laetrile suppliers submit evidence of any beneficial effects from the substance. The NCI has now analyzed the material submitted and reports its findings in the Sept. 7 NEW ENGLAND JOURNAL OF MEDICINE.

First the NCI sent letters to 385,000 physicians, 70,000 other health professionals and various pro-Laetrile groups requesting information about any cancer patients who purportedly had improved as a result of taking Laetrile. Although the NCI had hoped to receive 200 to 300 case histories to analyze, it got only 93, and of these only 67 had sufficient details to be scrutinized.

These 67 cases were then presented to a panel of 12 cancer specialists, along with an equal number of conventionally treated cases selected from the institute's files. The panel members were then asked to evaluate each patient's treatment progress, without knowing what treatment the patients had received. This way the NCI hoped to avoid charges of an anti-Laetrile bias.

Six of the 67 Laetrile patients were found by the judges to show complete disappearance of all evidence of cancer or shrinkage of tumors by 50 percent or more. The cancers involved were of the lung, lymph nodes, chest, abdomen and the gastrointestinal tract. Three more Laetrile patients were judged to have shown a longer survival than would normally have been expected for their form of cancer, although their cases were considered non-evaluable in terms of a measurable tumor response because Laetrile was used when no definite sign of disease was present. The cancers were of the testes, ovaries and lymph nodes.

For the remaining 58 cases presented to the panel, a total of 59 courses of Laetrile treatment were evaluated. (One patient was treated at two different times with Laetrile, and each course of treatment was evaluated separately.) Eleven of these treatment courses were judged to have insufficient data for evaluation, 32 as non-evaluable (either because the patient did not have cancer at the time Laetrile was given or because anticancer drugs were given along with Laetrile), nine as showing stable disease and seven as showing progressive disease.

"This retrospective analysis illustrates the difficulty of drawing inferences about the therapeutic efficacy [of Laetrile] in the absence of properly randomized trials," the NCI concludes. Nonetheless, results of the analysis will be presented on September 25 to a committee (composed of NCI physicians and scientists) that advises on the development of anticancer drugs. This committee will decide whether to recommend a clinical trial of Laetrile. The final decision will be made by NCI Director Arthur C. Upton, after consulting with FDA Commissioner Donald Kennedy and other authorities. □