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Editorial

Expanding the Uses of Enzymes

At the recent AAAS meeting in Boston a symposium organized by Rex Montgomery of the University of Iowa provided a glimpse of interesting areas where microbiology, biochemistry, and chemistry can be employed in a multidisciplinary approach to practical uses of enzymes. One thrust was efforts to convert food-processing wastes into higher value products. Another was applications of a spectrum of enzymes derived from various microorganisms to obtain useful substances. This approach sometimes involved reactions catalyzed by enzymes in media other than water. The topics treated in the symposium have long-term as well as near-term relevance, for gradually the world will become more dependent on products formed from feed stocks originally derived from plants.

Improving the value of agricultural products and dealing effectively with food-processing wastes are of special interest in Iowa and are well supported there. The multidisciplinary group of faculty scientists active in biocatalysis and bioprocessing research at the University of Iowa and the part of the Biotechnology Byproducts Consortium there is one of the strongest such groups anywhere. An example of the many objects of their research is corn oil. This lipid is rich in the 18-carbon oleic acid. Through microbial oxidation of the double bond, oleic acid can be converted specifically into hydrostearic or ketostearic acid. These can be employed as lubricants, surfactants, and plasticizers as well as in other ways. A wide variety of yeasts, fungi, and bacteria that can catalyze the oxidative reactions have been identified.

Lard, which consists mainly of fatty acids combined with glycerin, is another object of research at the University of Iowa. Many Americans now avoid a high-fat diet. Thus, demand for hald has fallen to the point where it has become a food-processing orphan by-product. A single abattoir in Iowa produces 150 million pounds of lard a year. What to do with it? One alternative is to dissect it into its components, one of which is the 16-carbon, straight-chain palmitic acid. An enzyme present in a strain of yeast cleaves only palmitic acid from lard. Other yeasts have enzymes specific for freeing remaining 18-carbon acids.

Enzymes can facilitate specific reactions of carbohydrates with other chemicals with a minimum of undesirable by-products. Sucrose contains eight potentially reactive OH groups. Ordinary chemical synthesis tends to lead to uncontrolled, undesirable cross-linking. When the appropriate enzyme is employed, one site only on the sucrose reacts with certain chemicals to form monomers that can be isolated and subsequently polymerized to make interesting, highly hydrophilic polymers. A variant of this procedure involves enzyme-catalyzed trans-esterification of monosaccharides with vinyl acrylate, with the reaction conducted in pyridine. The resultant esters are isolated and subsequently polymerized. The hydrophilic polymers are lightly cross-linked to form materials capable of holding 50 times their weight of water. The polymer would be largely biodegradable.

In contrast to the earlier conventional view, some enzyme-catalyzed reactions proceed better in nonaqueous media than in water. Advantages of using other media include increased solubility of nonpolar substrates, shifts in equilibria to favor synthesis over hydrolysis, suppression of water-dependent side reactions, and elimination of microbial contamination. To perform well in polar media, most enzymes seem to require the presence of 1 to 2 percent of water in the reaction mixture. Reactivity and selectivity of the enzymes in nonaqueous media may be improved by recombinant DNA protein engineering, which has been successful in enhancing catalytic activity of enzymes in water.

Microbiologists have long known that many microorganisms synthesize a wide range of compounds, using carbohydrates as the sole energy and carbon sources. Biotechnology companies such as Genencor International are exploiting these synthetic capabilities to obtain specialty chemicals. One of these is indigo, which is used for dyeing denim. In ancient times, indigo was obtained from plants. During the last century, it was synthesized chemically from toxic chemicals, including aniline, formaldehyde, and sodium cyanide. Through bioengineering, a strain of Escherichia coli has been created that produces substantial amounts of tryptophan, converts it to indole, and finally to indigo. Indigo produced by E. coli will soon be on the market competing with dye synthesized by the older industrial method.

Philip H. Abelson

*Biotechnology Byproducts Consortium, University of Iowa College of Medicine, 202 MAB, Iowa City, IA 52242-1101.
The Oberrothenbach Catastrophe

The article "A grisly archive of key cancer data" by Patricia Kahn (News & Comment, 22 Jan., p. 448) describes a catastrophe in which a large population in the region around Oberrothenbach in the former East Germany was exposed to excessive amounts of uranium and hazardous compounds produced by uranium mining. The manner in which this catastrophe is described implies that a natural experiment is in progress and that some interesting data will be obtained. Some epidemiologists are cited as referring to "the world's biggest treasure chest of data on radiation and human health" and "[a] treasure trove of data" (italics added). No medical practitioner with expertise in cancer or other disease prevention is quoted.

"How do we help!?"—not "How do we observe!?"—should have been the major theme of the article. Had a similar catastrophe occurred in the United States, the focus would not have been on observation, but on the need for assistance to exposed individuals by every means available.

Lee W. Wattenberg
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Kahn's excellent article about health statistics in the East German uranium industry sheds new light on an important and disturbing legacy of the East German nuclear effort. The same patterns of scientific censorship and callous disregard for miners' health occurred on the southern slopes of the Erzgebirge, in Czechoslovakia's famous uranium mines. A recently discovered secret agreement (23 November 1945) granted the Soviet Union exclusive rights to all uranium mined in Czechoslovakia and the mines around Joachimsthal were greatly expanded under Soviet supervision. Health records and vital statistics on the miners were coordinated by the state-run Health Institute of the Uranium Industry (HIUI) established in the mining town of Príbram in 1954 for this purpose. In 1960, Vladimir Reřicha, an epidemiologist at the HIUI, was asked to prepare an overview of the incidence of lung cancer among Czech miners; between 1960 and 1965 he and his staff compiled epidemiological evidence that 27,000 uranium miners at Joachimsthal and Horní Slavkov were dying from lung cancer at about five times the rate of coal miners and the male Czech population more generally, results that were in agreement with findings for U.S. miners at about this time (1).

Reřicha prepared an in-house paper detailing his findings for the HIUI in 1966 (2) and simultaneously sought to publish his results in a more conventional scientific journal. The report and its contents were classified by the State Security Police, however, and the ban was not lifted until the "velvet revolution" of 1989. As Reřicha recently recalled (3), Czech security authorities were apparently afraid that from uranium health statistics one could calculate either uranium production levels or the quality of uranium being mined, or both. The cynicism of such a ban was made apparent in the 1970s, when Reřicha was again refused permission to publish, despite the fact that the administrative chief responsible for Czechoslovakian uranium mining, Karel Boček, had defected to West Germany in 1970. With details of the nature and scope of Czech uranium mining no longer secret from the West, Czech authorities may have feared that revelation of the sacrifice of its miners for Soviet atomic power might not sit well with the Czech public.

Kahn notes that many East Germanuranium miners were forced laborers in the early years, but that coercion proved unnecessary, as food and work were in short supply and miners' wages were high. In Czechoslovakia, however, forced labor was an integral part of the uranium mining program for more than 15 years. The recently opened archives of the State Security Police show that the Czech government organized 17 forced labor camps at uranium mines. The number of political prisoners in the mines is recorded precisely in secret police archives, growing from 64 in 1946 (all Germans, presumably Nazis), to 5,500 in 1950 (all Czechs by this time), to a peak of 11,816 in 1953. All uranium mine political prisoners were released as part of a general amnesty granted in 1963; after this time, the only prisoners working in the mines were "ordinary" (that is, nonpolitical) criminals. Officials at the HIUI estimate (4) that, altogether, as many as 50,000 political prisoners may have worked in Czech uranium mines from the beginning of the camps in 1946 until the amnesty in 1963. Some of these prisoners continued working in the mines even after their release; civilian miners were paid very well—about ten times the average salary of physicians. Today, all Czech uranium miners are eligible for 60,000 kronen for health and hardship compensation; political prisoners became eligible for compensa-
Contamination of cDNA Sequences in Databases

We have evidence for heavy contamination of a large data set of human complementary DNA (cDNA) sequences in the nucleotide data libraries by sequences of an unknown prokaryote. We have retrieved from the databases 4888 putatively expressed human cDNA sequences that have been deposited recently from different human genome sequencing projects and have compared them [for a description of methods, see (1)] with the latest version of the SWISS-PROT protein database. The search showed that the largest of these collections of sequences [2366 entries in the European Molecular Biology Laboratory (EMBL) database as of 5 February], representing one set of cDNA clones derived from a T lymphoblastoid cell line, is heavily contaminated by prokaryotic sequences (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>cDNA library</th>
<th>Total sequences</th>
<th>Eukaryotic-like</th>
<th>Prokaryotic-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>T lymphoblastoid cell line</td>
<td>2366</td>
<td>120</td>
<td>278</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>356</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>291</td>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td>Fetal and adult brain</td>
<td>1875</td>
<td>386</td>
<td>1</td>
</tr>
</tbody>
</table>

The contamination is a major one involving more than 700 kilobases of human expressed sequence tags, of which at least 83 kilobases are of nonhuman origin. The contaminated sequences will remain in the data-base for the next few months, characterized as "human partial cDNAs." We propose that all sequences from the contaminated cDNA library except those that are clearly of human origin be moved from the "primates" section of the databases to the "unannotated" section.

References


References and Notes

1. The human cDNA sequences used for our search were retrieved from EMBL database release 33 and the EMBL daily updates until 5 February 1993. Sequences submitted by the Genexpress cDNA Program were selected by searching for the string GENEXPRESS in the author line (3013 entries from three cDNA libraries), and sequences from the United Kingdom/Molecular Research Council Human Genome Mapping Project were selected by searching for HSAAA as the first five characters of the entry name (1875 entries from two cDNA libraries). We used the program.
BLASTX (2) to compare these sequences (translated in six frames) with the protein sequences of the SWISS-PROT database release 24 on a silicon graphics cluster. The results were processed by taking the five best "hits" of each BLASTX output and filtering them to remove those with Poisson probability (Pval) greater than 10^-2. A sequence showing the lowest Pval with a eukaryotic protein was considered to be "eukaryotic-like," while a sequence with a top-matching prokaryotic protein was designated "prokaryotic-like." The complete results of this search are available by electronic mail.


Biotechnology in Japan

June Kinoshita, in her article "Is Japan a boon or a burden to U.S. industry's leadership?" (News, 29 Jan., p. 596), recounts a survey of Japanese pharmaceutical biotechnology that provides in some respects an update of a survey performed by the U.S. Food and Drug Administration (FDA) in 1988 (1).

Kinoshita cites a number of significant obstacles that prevent Japan from being a major competitor, but she does not mention that the regulatory climate in Japan has been, at best, equivocal toward new biotechnology. Japan has adopted a technique-based regulatory approach—with special requirements for products derived from recombinant DNA, and several areas have been significantly impeded. For example, despite a medical and scientific infrastructure that could support clinical trials of human gene therapy, no Japanese group is close to moving into the clinic, and no Japanese company has been created with gene therapy as its goal. By contrast, gene therapy trials are already under way in the United States, Italy, France, the Netherlands, and China, with almost 100 patients having been treated and the numbers rising exponentially (2).

Japan's attitude toward the new biotechnology is similarly reflected in agricultural biotechnology. Only a single field trial of a recombinant DNA-manipulated plant has been carried out in Japan (and none of microorganisms), and Japanese research and development in this area is behind what one would expect. The Japanese government has provided little encouragement in the form of clear, predictable, risk-based regulation to those contemplating field trials. Moreover, the Japanese Ministry of Health and Welfare has imposed a strict regulatory regime specific to foods and food additives manufactured with recombinant DNA techniques (3).

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References and Notes

Gene Therapy Approval Process

I would like to comment on several statements in the article "Harkin seeks compassionate use of unproven treatments" (News & Comment, 11 Dec., p. 1728) by Larry Thompson regarding a request by the San Diego Regional Cancer Center (SDRCC) that the National Institutes of Health (NIH) adopt a policy to expedite the review and approval of gene therapy protocols in cases involving terminally ill patients.

The central issue, all but lost in the article, is that NIH did not at the time have in place a policy to review and act on requests by terminally ill patients seeking the benefits of new gene therapy methods (1). The request was not a means of avoiding peer review but an attempt to streamline an existing process that in some cases literally exceeded the life expectancy of the patients seeking help.
The SDRCC request before the Recombinant DNA Advisory Committee (RAC) on 4 November dealt solely with this issue. A completely different SDRCC gene therapy protocol reviewed by the RAC year earlier was not under review at the recent RAC meeting, nor is it the basis of the gene therapy protocol under consideration for the patient who is discussed in the article.

Ivor Royston
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Notes

1. On 14 January, the RAC voted 9 to 3, with 1 abstention, to adopt an interim policy allowing internal NIH review and approval of genetic treatments for dying patients when the RAC cannot meet quickly enough to evaluate them (L. Thompson, News & Comment, 22 Jan., p. 452).

The Cost of Regulation

I was pleased to see that Philip H. Abelson’s editorial “Regulatory costs” (8 Jan., p. 159) made use in the first paragraph of my data on regulatory cost (1). I agree with the points Abelson makes and hope that his message—that the cost of regulation has mushroomed—receives broad acceptance.

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References


From the Vatican

In Constanza Holden’s article “Scientists’ campaign to save Earth” (News & Comment, 27 Nov., p. 1433), Henry Kendall, chairman of the Union of Concerned Scientists (UCS), is said to have claimed that the Pontifical Academy of Sciences has adhered to the USC’s campaign to save the Earth. As president of the Pontifical Academy of Sciences, I would like to say that this statement is not true. Any Pontifical academician who may have signed the UCS “Warning to humanity” has done so only in his own name.

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Spacecraft Neutrino. Christine Sutton. Cambridge University Press, New York, 1992. xiv, 244 pp., illus. $44.95; paper, $24.95.


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'Only a few meteorites have caused damage to dwellings. This photograph taken by Phil Dom- browski shows a recent occurrence: Wethers- field, Connecticut, November 8, 1982.' [From The Cambridge Guide to Astronomical Discoveries]