ATTACHMENT A

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McGRAW-HILL'S BIOTECHNOLOGY
NEWSWATCH
Genetic Engineering • Biomass • Patents • Enzymes • Energy • Monoclonals • Recombinant DNA

INSIDE in brief

Genentech readies trials for TPA, gamma interferon

SOUTH SAN FRANCISCO, CALIF.—Genentech, Inc., here will sell bulk human tissue plasminogen activator (TPA), a blood-clot-dissolving agent (*Newswatch*, June 20, p. 3), to Boehringer Ingelheim International, GmbH, of Ingelheim, German Federal Republic. Boehringer will handle regulatory approval and marketing of TPA in Europe, Australasia, the Middle East, Africa, and South America, says Genentech's Suzanne McKean. In the U.S., the firm is preparing clinical protocols for Food and Drug Administration permission to conduct the first human trials of recombinant-DNA-produced TPA. (See also p. 3.)

Genentech is also supplying interferon for the first U.S. clinical trials of r-DNA-produced gamma interferon. These began, *Newswatch* has learned, on Dec. 19 with one patient at M.D. Anderson Hospital and Tumor Institute of the University of Texas, Houston, and will soon start at the National Cancer Institute, Frederick, Md. (*Newswatch*, Dec. 6, '82, p. 3). Following regulatory approval, Genentech plans to market the interferon in the U.S. under its own label.

Meanwhile, Biogen NV, Geneva, Switzerland, together with Shionogi Pharmaceutical Co. Ltd., Osaka, Japan, began European and Japanese r-DNA gamma interferon clinical trials Sept. 15, and is now treating about 40 patients, says Seth Rudnick, Biogen's vice-president for medical research.

Viral vector, insect host glycosylate peptide at Texas A&M University

COLLEGE PARK STATION, TEXAS—A new and perhaps improved form of interferon cloned in a novel vector is now in preclinical animal trials. Entomologist Gale E. Smith of Texas A & M University hooked up the structural gene for beta interferon to the promoter sequence of an insect-virus gene, inserted the hybrid DNA message in a culture of insect cells and obtained expression of the protein.

Using the insect cell as a host organism and the insect virus as vector holds at least two genetic-engineering promises, says its inventor:

- ▶it produces a gylcosylated peptide similar to that synthesized in nature;
- ▶product yield is copious, thanks to the power of the gene promoter, which is designed to secrete its original viral protein in quantities up to half of the infected cell's mass.

In initial experiments, Smith tells *Newswatch*, he and his research team recovered five million units of interferon per million cells, which, he says, is in the order of magnitude of theoretical maximum yield, and 100 times better than recovery by previous methods using eukaryotic, animal or yeast, cells.

Eukaryotic cells, unlike genetically engineered bacterial hosts, glycosylate the peptides they produce.

Smith's beta interferon, synthesized in insect cells, is not only glycosylated but displays the same in-vitro activity in human cells as natural, glycosylated interferon.

"This is a significant achievement," states Dr. Patrick W. Trown, director of experimental and applied biology at Hoffmann-LaRoche, Inc., in Nutley, N.J. "We don't know if non-glycosylation in *E. coli* is a major problem," he tells *Newswatch*, "so ways such as this of producing it in glycosylated form are of great interest." But Trown adds, "What we don't yet know is if insect glycosylation is the same as human." He is responsible at Roche for preclinical testing of alpha, beta and gamma interferons.

Roche's cloned beta interferon, now in clinical trials, is made in cooperation with Genentech, Inc., of South San Francisco, which perfected the technique of synthesizing it in *E. coli* hosts.

Smith and his colleagues reported their discovery in the December 1983 issue of *Molecular and Cellular Microbiology*. He and Texas A & M are "considering patenting it," he says; the preclinical animal tests now under way in the U.S. are being conducted by a major pharmaceutical company, which he declines to identify.

Here is how the Texas team cloned their beta interferon in insect host cells:

▶they obtained the beta interferon gene from geneticist John Collins of the German Federal Republic's Institute for Biotechnology Research in Braunschweig-Stöckheim, and fused this structural DNA sequence to the promoter of a gene for polyhedrin, a protein abundantly synthesized by an insect virus.

▶This virus, *Autographa californica*, in nature preys on various insects that attack a variety of useful agricultural plants. So *A. californica* is well known and widely used by farmers as a bioinsecticide for control of field crops.

▶The protein that this double-stranded-DNA virus makes, polyhedrin, lodges in huge quantities as crystals in the cells of the insects it infects.

▶The insect whose cells Smith recruited as his recombinant host is the fall army worm, *Spodopteria frugiperda*, whose immortalized ovarian cells are available in culture. By inserting his hybrid gene into these cells, he got them to excrete glycosylated, free-standing beta interferon, unattached to any precursor or other protein, and to export it across the cell membrane.

In vivo, Smith explains, the polyhedrin gene—of which he harnessed the promoter to the beta-interferon coding sequence—is not needed by the virus to protect itself, as the protein crystals do in a living insect. Hence, it is readily fooled into making the alien, genetically engineered product instead.

"It's a very clever idea," says Dr. Samuel Baron, chairman of microbiology at the University of Texas in Galveston, "with the potential of producing large amounts of beta interferon very cheaply." But Baron, a leading authority on the interferons, says "it remains to be established that glycosylation represents a major advantage. Alpha interferon is made in bacteria," he points out, "and it doesn't need to be glycosylated."

Still, he tells *Newswatch*, "it could be extrapolated to other proteins where glycosylation is essential, and opens a lot of big opportunities—if, indeed, the right sugar groups are put in the right places."

Baron estimates the novel insect-viral method is "still a long way to scale-up. If they can get insect cells in quantity," he observes, "and develop adequate purification, full-scale production might come in three to five years—if they're extremely lucky."

Amgen claims 'first' in cloning erythropoietin, sees $100-million market

THOUSAND OAKS, CALIF.—Citing secret new gene-probing techniques, scientists at Amgen here report the first cloning and quantity expression of erythropoietin (EPO), the elusive human hormone, made mainly in the kidney, that controls red-blood-cell formation. Molecular biologist Fu-kuen Lin, who led the project, made the report to a recent meeting of Amgen's scientific advisory board.

A member of that board, biochemist Eugene Goldwasser of the University of Chicago, supplied Amgen with purified, native EPO extracted from human urine, which yields the protein in minute quantities.

"Until now," Goldwasser tells *Newswatch*, "we've been dealing with only microgram quantities of EPO. This development will make it possible to investigate things that could not be done before," notably the mechanism of erythropoietin action in the body.

And given more than three million patients with chronic kidney disease—which causes bouts of severe anemia—Amgen estimates the annual world market for its gene-spliced EPO at $100 million.

Dr. Philip Whitcome, the company's director of strategic planning, tells *Newswatch* that genetically engineering EPO proved a complicated problem "because there is not a good source of messenger RNA from human cells that produces the protein." He adds, "We had to take an unconventional approach."

This involved "a combination of proprietary techniques developed at Amgen, ranging from protein microsequencing and gene synthesis to novel [nucleic-acid] hybridization techniques. These enabled us to distinguish the correct gene sequence for EPO from other sequences that differed by only a single base change."

The work, Whitcome explains, "entailed hundreds of oligonucleotide probes." Conventional hybridization techniques, he points out, are limited to some 32 probes per incubation when scientists are searching for a specific stretch of DNA. But Amgen is now able to use more than 200 probes per cycle, "a really significant" accomplishment.

Because the Amgen probes are extremely sensitive, he says, the research team was able to target two very specific areas in the DNA of interest. "If both light up in the genome, you know you have the right gene," he notes. "It's a real tour de force to be able to fish out a single copy gene without a good source of mRNA to start with." The detected EPO gene represents less than one part per million in human DNA.

Whitcome declines to identify the cloning vehicle or host organism beyond saying the former involved known vectors "modified by Amgen to make it usable with the EPO gene." Nor would he indicate the yield of EPO expressed; the protein has a molecular weight of 40,000 daltons. The company has filed patent applications on the EPO product and many of the processes it developed for its manufacture.

Amgen is now setting up to do preclinical testing—characterization and purification of the protein, assay development, toxicity studies and eventually animal trials—before asking FDA to approve human trials of recombinant EPO.

"The first place to try it," suggests hematologist Jerry Powell of the University of Washington School of Medicine in Seattle, is to control anemia "in patients with chronic renal failure, and chronic inflammatory diseases such as arthritis, whose production of red

blood cells is sometimes compromised."

And Dr. Eben Feinstein, who directs the kidney dialysis unit at Los Angeles County-University of Southern California Medical Center, says the result of making erythropoeitin available in quantity "could be dramatic down the years." He points out that by reducing the number of blood transfusions to people with the severe anemia of renal disease, the danger of hepatitis infection and the fear of AIDS from contaminated donor blood could also be diminished.

U.S.-U.K. venture group invests $4 million in plant tissue-culture firm

GLASTONBURY, ENGLAND—A consortium of British and American venture capitalists has invested more than £2.5 million ($4.175 million) in Twyford Plant Laboratories Ltd. of this city. Known for its production of ornamental plants and flowers by tissue culture, with yearly sales of over £1 million, Twyford plans to exploit genetic engineering and other advanced breeding technologies to expand into vegetables, field crops and forest products.

David Leathers, an investment analyst with Biotechnology Investments Ltd. one of Twyford's British backers, tells *Newswatch*, "What has impressed us is that the company has proven technology combined with strong marketing capabilities and a strong team of scientists." Other United Kingdom investors include: Grosvenor Development Capital Ltd., Prutec Ltd. and the government's British Technology Group.

The U.S. money is coming from: Plant Resources Venture Fund, Princeton/Montrose Associates, New Enterprise Associates and F. Eberstadt & Co., Inc. The new infusion of capital will fund Twyford's three-year expansion plan, which involves doubling production capacity to make it the largest plant tissue-culture center in the world, setting up a new production facility in California at a site yet to be selected, and doubling the firm's research budget to exceed £500,000 annually. The funds will be mainly devoted to applying cellular biology and micropropagation with an eye to automating production. It will also provide capital for joint ventures with companies in the Middle and Far East to open new markets for date and oil palms.

"These palm projects are still at the negotiating stage," says Jeff Hooper, who left a plant-breeding subsidiary of Shell Oil to become Twyford's managing director. He will name only the Middle Eastern countries involved: Iran, Iraq, Jordan, Saudi Arabia and the United Arab Emirates. But Hooper notes that the research is well advanced, with 1,000 date-palm embryos, representing a score of species, rooting in the laboratory. Fifty plantlets have already been delivered to each of the countries named for outdoor field trials. "The potential market is enormous," says Hooper; more than 10 million date palms must be replanted every year.

Twyford's date-palm-cloning technique, says Dr. Ken Giles, the firm's research director, achieves somatic embryogenesis using meristem as the starting tissue.

Ciba scaling up TPA, leech protease inhibitor, in new biotechnology lab

BASEL, SWITZERLAND—Production of tissue plasminogen activator (TPA) by biotechnology will be the major project at Ciba-Geigy AG's new 43-million-franc ($20 million) laboratory just opened here. Prof. Jakob Nüesche, who heads Ciba's biotechnology division, says that Ciba plans to spend heavily in 1984 to scale up TPA output, either by cloning in microbes or by culturing human cells.

"The medical need for the product is large, Nüesche tells *Newswatch*, and we know what the molecule is going to do inside the human body, compared to—let's say—interferon, with its many pleiotropic effects." This knowledge provides an incentive for taking the rare clot-dissolving TPA through the expensive clinical trials ahead, he points out.

The TPA synthesis method Ciba settles on—bacterial cloning or mammalian-cell culture—will depend, Nüesche explains, on how important glycosylation of the native molecule turns out to be (see also story on page 1). "We just don't know how the genetically engineered material will work in practice inside a human being. The yeast product is also glycosylated, but not quite in the same way."

Nüesche expects to have synthesized enough material by late 1984 to start clinical trials. TPA, which has already been cloned by Genentech, Inc., of South San Francisco, is expected to save the lives of heart-attack victims by reopening clogged coronary arteries (*Newswatch, June 20, p. 3*).

Other therapeutic products to be cloned in Ciba's new laboratory, which has pilot-plant capacity of several thousand liters, includes the protease inhibitor eglin, which Ciba scientists think may be useful in treating emphysema and septic shock. Eglin, secreted in nature by leeches after attaching to their host, limits tissue destruction by the enzymes that break down "neutral" proteins, such as elastase in the lung, during microbial infection.

In the same way, it is thought this inhibitor can control the attack on body tissue by cathepsin G, an enzyme that bacteria release when they are lysed by macrophages during severe septic infections.

Yields of eglin cloned in *Escherichia coli* average 10 milligrams per liter of fermenter broth, and are "not a problem," says Dr. François Meyer, head of the project; scale-up is about to start. The problem, Meyer notes, will be "establishing the biological activity of the product in humans."

Further down the road to commercialization is a tissue-culture method for producing the sedative scopolamine from the plant *Hyoscyamus aegypticus*. Ciba's Dr. André Strauss is using air-lift fermentation to grow the suspended plant cells. Yields of the drug must be enhanced by a factor of one thousand, he points out, most likely by using protoplast mutation and somaclonal variation. But he does not rule out genetic engineering as a last resort.

PatentWatch

A listing of selected U.S. patents in the fields of biotechnology issued from November 22 to December 6, 1983.

PURPOSE, USE, OR PROCESS	U.S. PATENT NO. DATE ISSUED; DATE FILED	ASSIGNEE INVENTORS
BREWING: USE OF PLASMIDS TO CREATE KILLER YEASTS Strains of the yeast *Saccharomyces cerevisiae* containing plasmids from a killer strain of the yeast *Kluyveromyces lactis* exhibit antibiotic activity towards wild yeasts while resisting toxins produced by the other yeasts. New strain has "killer activity towards more kinds of yeasts" than previously known strains and prevents contamination by wild yeasts during brewing of sake. (See also *Newswatch*, Sept. 5, p. 6.)	4,418,150 Nov. 29, 1983 Feb. 18, 1982	Mitsubishi Chemical Industries Ltd., Tokyo, Japan Norio Gunge
CHEMICALS: BACTERIAL PRODUCTION OF XANTHAN GUM A novel strain of *Xanthomonas campestris*, unlike existing strains, produces xanthan gum "at high specific productivities . . . for several hundred hours without culture degeneration" during continuous fermentation on "inexpensive aqueous nutrient media." Product is useful in tertiary oil recovery; as a thickener in foods, pharmaceutical vehicles, cosmetics, and fracturing liquids; and as an emulsifying, stabilizing, and sizing agent	4,418,145 Nov. 29, 1983 July 14, 1980	Standard Oil Co. Chicago, Ill William P. Weisrock, Edward F. McCarthy
PARASITES: MONOCLONALS TO DIAGNOSE AND TREAT FLUKE INFECTIONS Use of monoclonal antibodies binding to fluke membrane glycoproteins to diagnose and treat active fluke infections in humans and animals, and to distinguish between acute and chronic infections. *Schistosoma* and *Fasciola* are "preferred targets"; schistosomiasis is "one of the most widespread and debilitating tropical infections."	4,416,866 Nov. 22, 1983 Aug. 28, 1981	The Johns Hopkins University Baltimore, Md. Mette Strand
HORMONES: METHOD FOR ISOLATING ENDORPHIN mRNA Isolation of endorphin mRNA from human tissues (brain, adrenal, pituitary, and pancreas) uses a synthetic, 15-base oligodeoxynucleotide as a "specific and sensitive" probe. Following reverse transcriptase synthesis and cloning of complementary DNA, endorphin, a neuropeptide potentially useful in slowing "movement of the gut, relaxing smooth muscle, and preventing pain," is produced by host bacterium.	4,416,988 Nov. 22, 1983 Sept. 17, 1982	None Harvey Rubin, San Diego, Calif
AMINO ACIDS: MICROBIAL PRODUCTION OF D-N-CARBAMYL-α-AMINO ACIDS Microorganisms obtained from hot springs hydrolyze hydantoins to D-N-carbamyl-α-amino acids, intermediates in the synthesis of D-amino acids, penicillins, and cephalosporins. These unnamed, gram-negative, thermophillic bacteria permit "more rapid enzymatic conversion" and "substantially higher substrate concentrations" than hydrolysis by *Pseudomonas*.	4,418,146 Nov. 29, 1983 July 28, 1981	BASF Aktiengesellschaft, Ludwigshafen, German Federal Republic Rolf Lungerhausen, et al.
IMMOBILIZED ENZYMES: SUPPORT MATRICES FOR BIOCATALYSTS Immobilized enzyme system consisting of porous, refractory inorganic oxide contacted sequentially with titanium tetrahalide, a diamine, and an enzyme solution. System is said to maximize opportunity for enzyme reactivity and to have "relatively high stability." The glucoamylase system, for example, is "of great commercial importance" because it has "at least twice the activity" of previous methods.	4,416,992 Nov. 22, 1983 Mar. 26, 1982	UOP Inc., Des Plaines, Ill. Blaise J. Arena, Ronald P. Rohrbach
VECTORS: PLASMIDS WITH BROAD HOST RANGE Novel plasmid rings suitable for use as cloning vehicles consisting of a fragment from pRP1 and DNA coding for desired protein, and carried in *Pseudomonas aeruginosa*. Unlike previously known plasmids, these have broad bacterial host range and a small size permitting efficient bacterial transformation	4,418,194 Nov. 29, 1983 Oct. 19, 1981	Microlife Technics, Inc., Sarasota, Fla. Ronald H. Olsen
VECTORS: BOVINE PAPILLOMA VIRUS AS A CLONING VEHICLE Novel vectors consisting of fragment of bovine papilloma-virus- (BPV) DNA capable of expressing eukaryotic and prokaryotic genes in eukaryotic cells. Cells transformed with BPV, which can be propagated indefinitely, contain 10-120 copies of the DNA, "grow faster . . . facilitating the large-scale production," and can be isolated visually without additional identification steps.	4,419,446 Dec. 6, 1983 Dec. 31, 1980	United States Department of Health and Human Services, Washington, D.C. Peter M. Howley, Nava Sarver, Ming-Fan Law
RECOMBINANT DNA: FUSED HYBRID GENE WITH BACTERIAL PROMOTER Fused hybrid gene consisting of a bacterial promoter containing a transcription initiation site (Shine-Dalgarno sequence) upstream from a protein translation start site (ATG) of a gene coding for a prokaryotic or eukaryotic protein (*Newswatch*, Dec. 19, p. 1). When cloned into bacteria for expression, it is "translated and transcribed efficiently."	4,418,149 Nov. 29, 1983 Feb. 5, 1982	President and Fellows of Harvard College, Cambridge, Mass. Mark Ptashne, et al.
DNA/RNA: REMOVAL OF BLOCKING GROUPS DURING SYNTHESIS Use of a primary amine with three to five carbon atoms, preferably a hinderec amine (particularly t-butylamine) in inert organic solvent (preferably pyridine) to selectively remove β-cyanoethyl blocking groups during synthesis of nucleic acids by the triester method. Process takes "a few minutes" rather than 4-6 hours as in previous methods.	4,419,509 Dec. 6, 1983 Aug. 24, 1981	Eli Lilly and Co., Indianapolis, Ind. Hansen M. Hsiung

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Ames, Beckman, DuPont compete in theophylline monoclonal assay kits

A MARKETING CASE STUDY

PHILADELPHIA, PA.—Yet another monoclonal-antibody-based serum theophylline drug monitoring assay entered the market this November. E. I. duPont de Nemours and Co., Wilmington, Del., makes the third entrant following Beckman Instruments, Inc., Fullerton, Calif., last fall, and Ames, a division of Miles Laboratories, Elkhart, Ind., this September. The three companies are relying on slightly differing strategies to carve out their share of a preexisting, one-million-test-per-year medical market. Theophylline—an alkaloid found in tea—is widely used in treatment of asthma. Because of its toxicity, serum levels of the drug must be continuously monitored. Existing polyclonal-antibody assays cannot distinguish between theophylline and caffeine or their metabolites, says DuPont's marketing manager for clinical and instrument systems, Frederick H. Fraser.

DuPont ties assay to machine

Newcomer DuPont is cashing in on a ready-made customer base—the 4,000 users of the company's automatic clinical analyzer (ACA), a computer-controlled system that can perform some 50 colorimetric and turbidometric clinical assays. The ACA system is widely accepted as the most reproducible around, claims Fraser, predicting, "We'll have a very significant share of the theophylline-testing market by mid-1984, since many hospitals and laboratories already have ACAs. ACA owners are already asking for the new test pack," Fraser tells *Newswatch*, "and there are a number of cases where competitive systems have been dropped in favor of DuPont's." Tying new reagents to existing state-of-the-art equipment "is definitely the way to go," he declares.

In the DuPont assay, serum theophylline competes for monoclonal antibody with a particle-bound theophylline analog, thus reducing antibody-particle cross-linking. The rate of particle formation, as measured by forward light-scattering, is inversely proportional to serum-drug concentration. Each test costs about $3.00 and takes seven minutes to run.

Beckman did it last year

Clocking in at only 80 seconds and $1.00 to $2.50 per assay, Beckman's year-old monoclonal theophylline assay has already captured 3% of the market, says Alfred J. Quattrone, principal chemist with the firm's Immunochemical Systems Group. Beckman's assay, like DuPont's, is tied to its proprietary detection equipment, the semi-automated ICS III or the automated Auto-ICS system. Quattrone estimates the ready-made base of ICS users at about 4,000 worldwide. Beckman markets four protein and eight drug assays along with its ICS equipment. The Beckman test is also an indirect inhibition immunoassay, using a particle-based detection system, but the company's equipment measures backscatter rather than forward scatter, a more accurate and sensitive method, claims Quattrone. Not so, responds Fraser. "Using DuPont's equipment, differences in forward light-scattering can give accurate readings down to less than one microgram per milliliter of serum."

Ames tries adaptable assay

Meanwhile, the Ames/Miles Laboratories' entrant uses a different detection system—a substrate-labeled fluorescent immunoassay—in its monoclonal-antibody theophylline kit, which takes 25 seconds to run and costs $1.50 to $1.20 per test. Ames marketing manager Jon Ruppert tells *Newswatch* that in addition to tailoring the kit for use in its own automated devices (a base of about 1,000 customers), Ames is marketing kits that can be adapted for use in other automated and semiautomated clinical testing devices and manual assay methods. This flexible approach should help capture a broader share of the drug-assay market, says Ruppert.

The theophylline-monitoring market has been dominated by the polyclonal-based assay kits produced by Syva Co., a subsidiary of Syntex Corp., Palo Alto, Ca., and Abbott Laboratories, N. Chicago, Ill., which also tie their diagnostic reagents to automated systems. Are they running scared since these monoclonal test kits reached the market? Comments Abbotts' divisional director and vice-president of research and development, David V. Milligan, "The mere mention of monoclonal antibodies these days causes investors' ears to perk up. We intend to introduce monoclonal-based products but regard them as just one arrow in our quiver." In clinical assays, Milligan adds, "you need a broad array of diagnostic tools. Monoclonal antibodies haven't proven to be the be-all and end-all."

Morinaga lung antibody still on drawing-board

TOKYO—Using an immortalizing cell line that grows in non-serum culture medium, Morinaga and Co. Ltd. here is developing low-production-cost all-human, monoclonal antibodies which they describe as targeted against lung cancer. Through its subsidiary, Morinaga Biochemical Research Laboratory (MBRL) in Yokohama, this is the medical diagnostics firm's first venture into pharmaceutical products.

Dr. Hironori Murikami, professor of food and chemical engineering at Kyushi University, initially developed the parental lymphocyte cell line that multiplies in serum-free culture medium. The high cost of serum is an economic barrier to commercialization of human cell culture. Now MBRL researchers are fusing this parent stock with cells collected from lymph nodes of lung cancer patients, and selecting those hydridomas that produce antibodies to pulmonary tumor-associated antigens.

But so far these antibodies have exhibited broad affinities toward various types of malignancy, reacting in vitro with prostate and breast-cancer cell lines and malignant melanomas as well as pulmonary neoplasms.

"I see nothing new in it," says Carlo Croche of the Wistar Institute in Philadelphia. "Attempts to establish antibodies to specific organ-related cancer cells have gone on for years in this country."

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MEETINGS • SYMPOSIUMS • COURSES

PLACE	DATES	THEME	SPONSOR
Miami Beach, Fla.	Jan. 16-20	**Advances in Gene Technology: Human Genetic Disorders** (symposium topics: oncogenes, chromosome instability, gene activation, genetic disorders, diagnosis and therapy)	Miami Winter Symposium P.O. Box 016129 Miami, FL 33101 Att: Sandra Black Telephone: (305) 547-6265
Tucson, Ariz.	Jan. 23-25	**Biochemistry of the Reproductive Years** (conference includes sessions in embryo transfer and in vitro fertilization)	American Association for Clinical Chemistry 1725 K St., NW, Suite 903 Washington, DC 20006 Telephone: (202) 857-0717
Lake Buena Vista, Fla.	Jan. 30-Feb. 3	**Energy from Biomass and Wastes, Eighth Annual Symposium** (topics include: genetic engineering advances, cellulose-to-alcohol conversion, commercialization, regulations, economics)	Institute of Gas Technology 3424 S. State St., IIT Center Chicago, IL 60616 Telephone: (312) 567-3881 Att: Ms. Maryann Manrot
New Delhi, India	Feb. 19-25	**VIIth International Biotechnology Symposium** (recombinant DNA, plant and animal cell culture, hybridomas, scale-up, biometallurgy, chemical feedstocks, fuels, immobilized enzymes and cells, separation, and process engineering)	Biochemical Engineering Research Centre Indian Institute of Technology, Delhi New Delhi, 110 016, India Att: Symposium Secretariat Telephone: 651880; 654110
Philadelphia, Pa.	Feb. 28-March 1	**Mechanisms of Receptor Regulation, Research Symposia II** (cell surface receptors, ligand interactions, signal transduction, processing, recycling, modulation and genetic approaches to regulation)	Smith Kline & French Laboratories P.O. Box 7929 Philadelphia, PA 19101 Att: Dr. George Poste Telephone: (215) 751-3157
London, England	March 18-20	**Biotechnology in Oil Production** (course topics: polymers and surfactants, plugging and permeability, desirable vs. undesirable species in oil-bearing strata, logistics of R & D)	School of Biological Sciences Queen Mary College London, E1 4NS, UK Att: Prof. V. Moses Telephone: 01-980 4811, ext. 572
Columbia, Mo.	March 19-21	**Gene Manipulation in Plant Improvement, 16th Stadler Genetics Symposium** (interspecific hybridization, chromosome, chloroplast and mitochondrial manipulations, tissue culture, nitrogen fixation, somoclonal variation, gene expression)	Stadler Genetics Symposium Curtis Hall, University of Missouri Columbia, MO 65211 Att: Dr. J.P. Gustafson Telephone: (314) 882-7318
Piscataway, N.J.	March 19-21	**Molecular Cloning Workshop** (presented by Dr. Pieter C. Wensink, The Rosenstiel Medical Sciences Research Center, Brandeis University)	Waksman Institute of Microbiology P.O. Box 759 Piscataway, NJ 08854 Att: Director, Continuing Education
Rockville, Md.	March 19-21	**Freezing and Quality Control: Cell Cultures and Hybridomas** (hands-on workshop for those who need to preserve cell cultures and hybridomas reliably)	American Type Culture Collection 12301 Parklawn Drive Rockville, MD 20852 Att: David Grounds Telephone: (301) 881-2600
Cambridge, Mass.	April 9-11	**Biotechnology of Marine Polysaccharides** (seminar topics: biofouling; production and use of carrageenan, chitan and chitosan in pharmaceuticals, foods, feeds and chemicals, regulatory problems)	Massachusetts Institute of Technology, Sea Grant Program 77 Massachusetts Ave., Bldg. E38-302 Cambridge, MA 02139 Att: Therese Z. Henderson Telephone: (617) 253-7041
Urbana-Champaign, Ill.	May 14-18	**Plasmids in Bacteria** (conference topics: plasmid structure, evolution, replication, incompatibility, partition, transfer and specialized functions)	The University of Illinois at Urbana-Champaign Council for Research Planning in Biological Sciences Urbana, IL 61801 Att: Edna Unfer Telephone: (217) 333-2883
London, England	May 15-17	**Biotech Europe** (conference topics: call for papers in 25 areas of biotechnology; industrial exhibition)	Online Conferences Ltd. Pinner Green House Ash Hill Drive Pinner HA5 2AE, UK Telephone: 01-868 4466
Gatlinburg, Tenn.	May 15-18	**Biotechnology for Fuels and Chemicals** (symposium topics: genetic engineering and biomass conversion, bioprocess engineering, monitoring and control systems)	Oak Ridge National Laboratory P.O. Box X Oak Ridge, TN 37830 Att: Charles D. Scott
Conroe, Tex.	May 21-25	**Microbial Enhancement of Oil Recovery** (conference topics: field applications, economics and environmental concerns)	Energy Resources Institute University of Oklahoma Norman, OK 73019 Att: Mrs. Josephine L. Wilke Telephone: (405) 360-1600

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Japan seeks advantage in high-polymer membranes to separate bioproducts at low cost by 1990

TOKYO—Synthetic membranes, which separate fermentation liquids or gases that distillation cannot easily purify, brought a nine-member Japanese mission to the United States and Western Europe last month. Team leader Koso Mima of Toray Industries, Inc., here tells *Newswatch* that the two-year-old Research Association of Polymer Basic Technology (RAPBT) arranged the trip to "investigate up-to-date technology of high-polymer membranes that will cut the energy cost and improve specificity of bioproduct recovery." Mima declines to divulge the finding of the recent trip on the grounds that he has yet to make his report to RAPBT executives.

The member companies expect marketable membranes by the 1990s. Unlike present-day semipermeable barriers that act like sieves, ultra-thin polymer membranes are being designed to separate substances with similar boiling points, those which distillation cannot separate. They are also working on affinity membranes that will allow desirable biomaterials to pass through at high energy savings. Typical applications: ethanol recovery and removal of toxins in wastewater treatment.

The research was initially financed by the Ministry of International Trade and Industry (MITI) with a $1-million equipment grant in October 1981 to companies in the membrane-research consortium, and double that sum last year. RAPBT's master plan is to begin "trial production of things in 1985," says a spokesman who requests anonymity. The Japanese are guarding their information closely; association members have already filed 33 patents at home, but none abroad, according to a patent manager from one of the nine companies. "Perhaps 10% to 20% of these will be commercialized," he adds. Even laboratory equipment and supplies have been purchased almost exclusively in Japan.

MITI, which is sponsoring the entire thin-membrane project, will take title to all patents, with inventor companies manufacturing products under license, *Newswatch* understands. The participating firms are said to have invested $5 million in the national project and assigned to it about 50 researchers.

RAPBT members interested in liquid separation include:

- ▶Daicell Chemical Industries Ltd. of Sakai-shi;
- ▶Kuraray Co. Ltd.,
- ▶Sumitomo Electric Industries Ltd. and
- ▶Toyobo Co. Ltd., all of Osaka;

In the gas separation group are:

- ▶Asahi Glass Co. Ltd.,
- ▶Mitsubishi Chemical Industries Ltd. and
- ▶Toyobo Co. Ltd., all of this city; and
- ▶Asahi Chemical Industry Co. Ltd. and
- ▶Teijin Ltd., both of Osaka

Three government organizations collaborating with RAPBT are:

- ▶National Chemical Laboratory for Industry,
- ▶Research Industry for Polymers and Textiles,
- ▶Industrial Products Research Institute.

X-ray crystallography aids Agouron enzyme redesign

LA JOLLA, CALIF.—Using the knowledge gleaned from x-ray crystallography, the Agouron Institute here is putting new predictability into protein engineering. Its scientists are using highly detailed three-dimensional enzyme structures to decide how and where genes coding for proteins should be altered. (See *Newswatch*, Sept. 5, p.8.) Such proteins might be modified to work at high or low temperature and pH—conditions that normally make them unusable.

The first progress they report is customizing the enzyme dihydrofolate reductase (DHFR) (*Science*, Nov. 18). This ubiquitous enzyme plays a catalytic role in the metabolism of one-carbon compounds used in the biosynthesis of thymidylate, purines and some amino acids. It is also the target of a class of widely used cancer drugs, including methotrexate. The team, led by molecular biologist J. E. Villafranca, directed specific amino acid changes in three chosen areas through site-specific mutagenesis, and thereby planned precise alteration of the nucleotide sequence coding for the protein. One change involved the enzyme's active site; by changing an aspartate residue to asparagine, they reduced the enzymes's catalytic activity by 99.9%.

"Before, we could only make educated guesses about how altering one amino acid in an enzyme might modify the structure and function," says Villafranca. "But the combination of the very high resolution picture of an enzyme provided by x-ray crystallography and site-specific mutagenesis enables us to understand and to manipulate the crucial relationship between its structure and function."

But Peter Johnson, chief administrative officer of the institute, admits that this approach is not readily applicable to many enzymes because of the arduousness of the x-ray method. "Of the thousands of protein molecules, the three-dimensional structure of only approximately 200 are known. And perhaps fewer than 10 of these are known to the two-angstrom level of resolution, which is required to do precise protein engineering," he tells *Newswatch*.

The non-profit institute does not expect any direct commercial applications for the DHFR enzyme, but sees it rather as a model system. However, says Johnson, Agouron is investigating several other enzymes—which he declines to name—that have more "intrinsic and immediate applications." He adds that the institute has held discussions with companies on such protein engineering projects. Although the basic techniques are well established, and therefore not patentable, he believes that patents on the tailored enzymes will be possible. "Ultimately it should be possible to engineer enzymes from scratch for specific catalytic tasks," he speculates.

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14 MIT scientists share Grace microbiology grant

CAMBRIDGE, MASS.—Fourteen research projects in microbiology at the Massachusetts Institute of Technology here will share the first grants from a fund set up for the purpose by W. R. Grace & Co. of New York. Established in July 1982, the Grace fund provides MIT with $6 million to $8.6 million for microbiology research over five years. The grants are awarded annually for projects selected by a joint committee representing the university faculty and the chemical company's management.

This year's 14 projects and their research directors are:

►Biospecific adsorption with immunoadsorbents for isolation of biological compounds.
Clark K. Colton

►Microbial production of serine.
Charles I. Cooney

►Overproduction of threonine, and enzymatic synthesis of specific dipeptides.
Arnold L. DeMain

►Enhancement of selective immunity to infectious agents.
Malcolm L. Gefter

►Isolation of yeast nuclear cytochrome genes.
Leonard P. Guarante

►Liquid-liquid extraction of biopolymers.
T. Alan Hutton

►Cloning *Caenorhabditis elegans* genes by purification of DNA from free duplication.
H. Robert Horvitz

►Enzymatic separation of racemic mixtures of hydroxy compounds.
Alexander M. Klibanov

►Structural basis of protein stability.
Gregory A. Petsko

►Thermal stability of proteins.
Robert T. Sauer

►Construction and production of active enzyme fragments, and polyproteins with multiple activities.
Paul R. Schimmel

►*Corynebacterium glutamium.*
Anthony J. Sinskey

►Plasmid biology.
Graham C. Walker

►New concepts in bioreactor operations.
Daniel I.C. Wang

Biofuturism modeled at Battelle scale-up meeting

COLUMBUS, OHIO—With one exception, there were no surprises at last month's symposium on bioprocess scale-up at the Battelle laboratories here. Some 150 specialists, mostly from industry, reviewed with two dozen experts the present state and future prospects of recovering more and better biosynthesized products from genetically modified or unconventional microorganisms and plant and animal cells.

The one surprise came when biologist Gordon H. Sato, director of the W. Alton Jones Cell Science Center in Lake Placid, N.Y., began his talk on "Future Trends in Cell-Culture Bioprocessing." Citing as his futurist sources back issues of the *National Enquirer* plus his own laboratory crystal ball, he predicted:

"Early in the 21st century, a Polish mathematician working in Israel will develop a new brand of mathematics. A Chinese-American engineer in Cambridge, Mass., will apply this to non-Newtonian rheology. A Dutch biologist will apply these new mathematical models to reactor design to develop the first million-liter bioreactor, with totally efficient mass transfer.

"However, a Swiss genetic engineer in a pharmaceutical company will develop novel methods of gene amplification that increase the rate of production of desired molecules one-thousandfold.

"This technology is mysteriously transferred to a Japanese soya sauce company, and results in the miniaturization of bioreactors such that a one-liter vessel can produce the world supply of bio-reactive molecules.

"Thousands of biologically active clinically useful molecules undreamed-of now will be produced, leading to such an understanding of physiology that all diseases are eradicated except for one recalcitrant one that resembles what we now know as aging—except that typical onset occurs in the second century of life.

"A German algologist, using genetically-engineered unconventional organisms, will fill the deserts with green ponds. An Indian microbiologist working in a French-Canadian brewery will convert these to fuel and feedstocks. Basic commodities will be produced in such abundance that international tensions will be eased, leading to an unprecedented era of world peace. . . ."

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