JAPAN ROUNDUP

Novo Yakuhin K.K. (Tokyo) will import human insulin into Japan for use in the treatment of diabetes, now that it has received approval from the Ministry of Health and Welfare. The preparations to be used are Monotard Human and Actrapid Human, an intermediate- and short-acting preparation, respectively.

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Teams at both the Aichi Cancer Institute's Laboratories of Cell Biology and U.S. National Institutes of Health (NIH) were successful in the first cloning of the genes coding for DNA polymerase beta, an enzyme that synthesizes DNA and possibly could repair chemical damage.

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The Ministry of Agriculture, Forestry and Fisheries will begin joint R&D studies on biotech-aided crop breeding technology with U.S., West German, and French companies. The 10-million-yen budget for fiscal 1986 will fund research on recombinant DNA technology, cell fusion, and tissue culture as they apply to crop breeding.

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Mochida Pharmaceutical Co. (Tokyo) is the first company to develop urinastatin, a new biologically active substance, from human urine. It is marketing the agent under the trade name "Miraclid," for treating shock and pancreatitis. The drug is a multivalent enzyme inhibitor which, the company says, could achieve sales of up to 3 billion yen.

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The Institute of Physical and Chemical Research (Tokyo) will begin the operation of its Gene Bank in Tsukuba in 1987. The bank expects to supply over 100,000 genes and some 10,000 species of cells to researchers over ten years. It will also act as a national center of information on genetic science in cooperation with the U.S. and European countries.

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Dainabbot Co. Ltd. (Tokyo) is seeking approval from the Ministry of Health and Welfare to import the AIDS diagnostic radioimmunoasay test kit made by its U.S. parent, Abbott Laboratories (Chicago, IL).

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Eli Lilly Japan (Kobe) has begun the first Japanese clinical trials of *Escherichia coli*-produced human growth hormone with the N-terminal methionine removed.

RESEARCH PAPER ANALYSIS

A NEW TWIST IN BETA-LACTAM BIOSYNTHESIS

CAMBRIDGE, Mass.—The findings of Juan-Francisco Martín and coworkers at the University of León reported in this issue are completely unexpected. They have shown that the cyclase of certain high-antibiotic-producing fungi is able to convert a phenylacetate-containing linear peptide directly to benzylpenicillin.

Prior to this report, it seemed rather clear that *Penicillium chrysogenum* and *Acremonium chrysogenum* (*Cephalosporium acremonium*) share the initial steps of beta-lactam biosynthesis—the formation of the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine from L-α-aminoadipate, L-cysteine, and L-valine followed by its cyclization to isopenicillin N—and then part ways. Whereas *Penicillium* exchanges the L-α-aminoadipyl side chain for a hydrophobic one (e.g. phenylacetyl in the case of penicillin G), *Acremonium* epimerizes the side chain to the D-form (i.e. to form penicillin N) and then expands the thiazolidine ring of the penicillin to yield deacetoxycephalosporin C.

Studies on the substrate specificity of the cyclization enzyme (isopenicillin N synthetase; cyclase) have been done by a number of groups, including Baldwin and Abraham (Oxford), Jensen and Westlake (Alberta), Wolfe (Queens University), and Demain (M.I.T.). These showed that there is considerable flexibility in the third position (i.e. D-valine) of the tripeptide, no flexibility in the second (L-cysteine) position, and only some flexibility in the first (L-α-aminoadipic acid) position. Thus, although L-carboxymethylcysteine, adipic acid, or D-α-aminoadipic acid can replace L-α-aminoadipic acid in the first residue of the tripeptide, the following cannot: L-glutamic acid, L-aspartic acid, caproic acid, delta-aminovaleric acid, glutaric acid, aminopimelic acid, and acetic acid. Indeed, Baldwin et al. (*J. Chem. Soc. Chem. Commun.* 1225–1227, 1984) recently concluded that the first residue of the tripeptide must have a six-carbon or equivalent chain terminating in a carboxy group to be converted to penicillin. Thus, the direct conversion of the tripeptide phenylacetyl-L-cysteinyl-D-valine to penicillin G by *Cephalosporium* is very surprising. Indeed, early in cephalosporin research, a number of attempts were made to force *Cephalosporium* to produce phenylacetyl-cephalosporins by feeding phenylacetic acid to the culture, but all failed—presumably because *Cephalosporium* lacks the penicillin acyltransferase enzyme thought to be necessary.

The commercial implications of this discovery are unclear. Although the extent of the conversion appears to be very low, it is significant and does show that the cyclase of both *P. chrysogenum* and *A. chrysogenum* has affinity for the analog peptide. Even if it becomes possible through genetic engineering techniques (e.g., *in vitro* mutagenesis) to increase that affinity, it would only result in *P. chrysogenum* having an alternate pathway to form its normal antibiotic, penicillin G, and in *A. chrysogenum* having the ability to produce penicillin G along with its normal extracellular co-products, penicillin N and cephalosporin C. Only if the ring-expansion enzyme (deacetoxycephalosporin C synthetase; expandase) of *A. chrysogenum* could also be genetically modified to accept a hydrophobic penicillin as substrate would a hydrophobic cephalosporin be produced. Such a cephalosporin would be much easier to isolate from broth than cephalosporin C and would markedly decrease the cost of cephalosporins. Up to this point, the expandase has been shown not to act on hydrophobic penicillins.

Despite the lack of obvious immediate commercial utility, the observations of Martín et al. are very important. They suggest that the specificities of important biosynthetic enzymes are not necessarily as narrow as previously thought. This should give encouragement to the proponents of enzymatic biosynthesis (Wolfe et al., *Science* **226:**1386–1392, 1984) as a means of making new antibiotics as well as replacing expensive chemical steps in the preparation of current semi-synthetic penicillin and cephalosporins.

—Arnold L. Demain