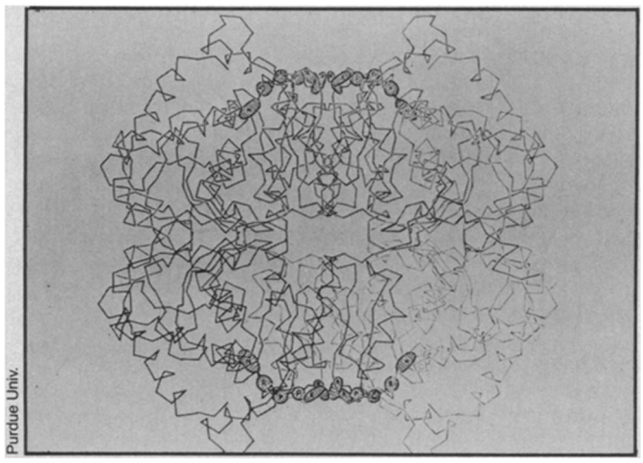
Enzymes: Medicine's New Gold Mine

Thanks to recent advances in technology, enzymes are being used as drugs, in the production of drugs and in the prevention of disease — and there's more to come



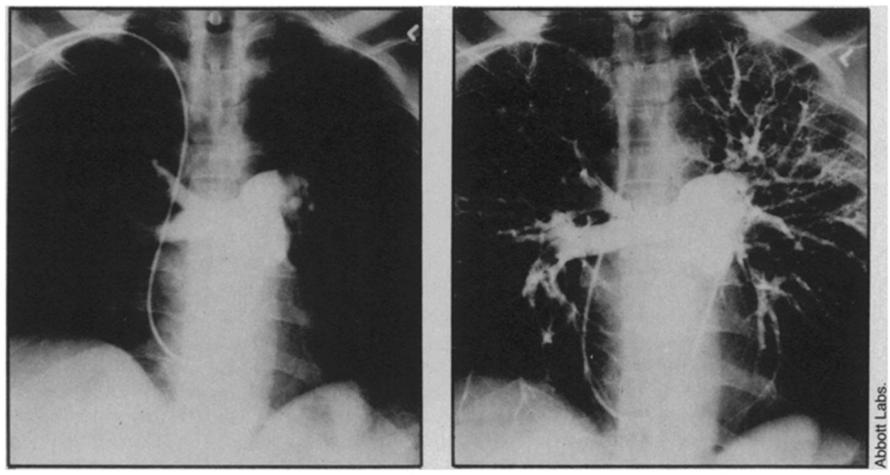
Computer drawing of lactate dehydrogenase showing only the main chain of each subunit (see cover photo).

BY JOAN AREHART-TREICHEL

It started with tens of thousands of gallons of urine collected from army and navy latrines. It ended with the marketing of a drug to treat blood clots of the lungs. And it is but one example of an exciting new era in clinical medicine: Enzymes — cellular chemicals that speed up reactions without taking part in them — are being used more and more as drugs, in the production of drugs and in the prevention of disease.

In the 1950s an enzyme called urkinase (found in human urine) was reported to successfuly dissolve blood clots. Drugs available at the time could only prevent clots from getting larger or prevent new ones from forming. To test the superiority of urokinase over existing drugs, researchers at Abbott Laboratories in Chicago extracted the enzyme from large collections of urine and helped the National Institutes of Health conduct clinical trials on patients with lung clots — a condition that kills up to 150,000 persons in the United States each year. The trials, which took place in the late 1960s and early 1970s, proved urokinase to be highly effective in dissolving clots that cause massive blockages of lung circulation. Meanwhile, Abbott researchers developed a method of extracting urokinase from cultured kidney cells — faster and cheaper than from urine. Urokinase from kidney cells was approved by the U.S. Food and Drug Administration this February, and by March Abbott was marketing it as a drug to treat lung clots.

Things are beginning to happen rapidly in the enzyme field because of progress made during the past decade or so. For one thing, as urokinase production illustrates, enzymes can now be produced in large batches from cultured cells. For another, "It took a hell of a long time," as one enzyme scientist recalls, to learn how to purify intracellular enzymes, and it's the intracellular enzymes that are primarily of interest to clinical medicine, versus extracellular enzymes — those made inside cells, then excreted — and have been used for some time in detergents, fermentation, cheese production, tanning and some other commercial operations. The technique that finally made intracellular enzyme purification economically possible



Angiogram (l.) shows decreased blood flow due to massive embolism. Two hours after urokinase treatment dramatic change is seen in blood flow through both lungs (r.).

was affinity chromatography (which makes use of a compound's biological activity). One reason that enzymes have come to be valuable in drug production and also in disease prevention is that they can now be immobilized on supports (chemically bonded to an insoluble material) and used again and again to manufacture, transform or break down select chemicals. Finally, research into enzymes' value to clinical medicine has been fueled by finances from the National Science Foundation's RANN (Research Applied to National Needs) program.

What enzymes other than Abbott's urokinase are now emerging as drugs? Streptokinase, an enzyme produced by *Streptococcus* bacteria in culture then purified by affinity chromatography, has also been approved by the FDA for dissolving lung clots. It was okayed before urokinase, in fact, in late 1976, and is being marketed by the American Hoechst Corp. Sterling-Winthrop Laboratories is also trying to get FDA approval to market urine-derived urokinase, and Abbott Laboratories is continuing clinical studies, as is NIH, to see whether urokinase can dissolve heart clots as well as lung clots. Novo Industri A/S in Copenhagen, the world's largest producer of enzymes for commercial use, is deploying lactase enzymes to break down lactose in milk so that the milk can be drunk by lactase-deficient people. Milk intolerance due to lactase deficiency is a major health problem.

"Quite a few American drug companies are now interested in enzymes as drugs," asserts Michael Weibel, a scientist with Novo Laboratories in Wilton, Conn., a division of Novo Industri A/S.

"I think drug companies will be getting more interested in enzymes as drugs," predicts Tom Craig, a spokesman for Abbott.

"Yes, enzymes as drugs are a new area," concurs David Price, a spokesman for Baxter Travenol Laboratories in Deerfield, Ill.

Enzyme drugs are also emerging from university labs. For instance, the itchy rash of poison ivy, poison oak and poison sumac is due to a toxic, oily irritant called urushiol. It is estimated that up to 70 percent of the U.S. population is sensitive to

urushiol. Although various poison ivy, oak and sumac preventive medicines are currently on the market, none has been shown to cleave urushiol into a nontoxic product without affecting chemicals in human skin as well. Now David P. Borris, a chemist at the University of Mississippi in University, Miss., has isolated several enzymes from bacteria that can perform this selective feat, that is, quickly transform urushiol into a nontoxic product either before or after a person's skin has come into contact with it. He has patented these enzymes as poison ivy, poison oak and poison sumac drugs.

Julian L. Ambrus and Clara Ambrus, physician-scientists at Roswell Park Memorial Institute in Buffalo, N.Y., have purified enzymes that destroy the amino acid phenylalanine, attached the enzymes to artificial blood vessel bypasses and passed monkeys' blood through the bypasses. Enzymes on the bypasses destroyed phenylalanine in the monkeys' blood. The researchers foresee a similar technique being used to treat children with phenylketonuria, a condition that can result in mental retardation if the excessively high levels of phenylalanine in the blood are not removed.

Univ. of Miss.

Borris has patented enzymes as drugs to treat poison ivy, oak and sumac.

As for enzymes in drug production, E. Kendall Pye, a biochemist and enzyme authority at the University of Pennsylvania School of Medicine in Philadelphia, and his colleagues have used two enzymes to make a drug that looks promising for dissolving gallstones, a common medical problem now only correctable by surgery. The drug is called chenodeoxycholate. What Pye and his co-workers did, essentially, was grow organisms known to produce the enzymes, recover and purify the enzymes, attach the enzymes to supports, then pass cholic acid through the supports. The supports help retain the enzymes in a fixed position and improve their stability. The enzymes converted cholic acid to chenodeoxycholate. This method of drug production contrasts sharply with the usual one — organic chemistry synthesis, in which different chemicals are reacted with one another.

Still another means of making drugs quickly and cheaply, compared with the usual chemical synthesis route, would be to immobilize microbial cells containing enzymes of interest, rather than the enzymes themselves, then to pass chemicals through the cells. Enzymes in the immobilized cells would then transform the chemicals into the desired drug. This tack, being explored by W.R. Veith of Rutgers University in New Brunswick, N.J., would eliminate the time-consuming and expensive steps of purifying intracellular enzymes and attaching them to supports. Enzymes in their natural environment — cells — should also be more stable than enzymes extracted and then used for drug production.

Univ. of Penn.

Pye: Producing drugs with enzymes rather than by the usual organic synthesis.

As for enzymes in disease prevention, one of the more interesting applications is the use of enzymes to filter pathogens from air as it moves through air conditioners. John Gainer and his chemical engineering team at the University of Virginia in Charlottesville were interested in using enzymes to remove pathogens from water. The enzymes worked, but not as economically as the researchers had hoped. They then explored the feasibility of using enzymes to filter pathogens out of air before it enters air conditioners. They purified enzymes known to destroy bacterial and viral genes or to rip off viral protein coats and immobilized them chemically to fiberglass or to foam filters in air conditioners. Sure enough, the immobilized enzymes destroyed the desired pathogens before they passed through the air conditioners.

Gainer and his co-workers have patented this system in the United States and five other countries and are currently talking with companies about manufacturing and marketing it. As Gainer told SCIENCE NEWS, "We think the system will be most beneficial for people in hospitals or office buildings where air circulates in a closed environment, although it might be used in homes as well."

Some of the most valuable medical uses for enzymes are just getting underway — for instance, deploying them to correct enzyme deficiency diseases. The first real coup in this area was reported in late 1976 by Stephen H. Polmar and his colleagues at Case Western Reserve University School of Medicine in Cleveland. They injected red blood cells loaded with the enzyme adenosine deaminase into an infant suffering from severe combined immunodeficiency disease and lacking adenosine deaminase. Enzymes in the red cells helped restore the patient's immune system and enabled him not only to survive but to lead a relatively normal life (SN: 1/1/77, p. 4).

Red cell enzyme therapy has some serious drawbacks, however. Transfused red cells have a maximum lifespan of only a few months and are capable of causing hepatitis or an iron overload. What enzyme researchers would rather do is inject purified intracellular enzymes into patients to correct their enzyme deficiencies. Such an effort, in fact, was launched for the first time in 1973 and 1974 by Roscoe O. Brady and his colleagues at the National Institute of Neurological and Communicative Diseases and Stroke in Bethesda, Md. They purified enough enzymes to treat two patients with Fabry's disease and two patients with Gaucher's disease. Both diseases are due to the inheritance of too few fat-metabolizing enzymes, which in turn leads to an abnormal accumulation of fats and causes serious body damage. The therapeutic strategy

Brady and his co-workers used was to give these patients normal versions of their abnormal enzymes. Before then, there was no effective treatment for these seriously debilitating diseases.

This effort was hailed as "an important and exciting advance" by an editorial in the Nov. 7, 1974 New England Journal of Medicine. But as the subsequent four years have shown, perfecting the treatment has not been as easy as Brady and his colleagues had hoped. As Brady explained to Science News recently, "The enzymes seemed to correct some of the biochemical problems of these patients, but they did not produce the clinical improvement we expected to find."

Nonetheless, Brady and his team are optimistic that they can still make enzyme replacement work. "I think we know what the main problem is," Brady attests, "and we are trying to correct it. The enzymes are probably not going to the right cells fast enough, so we are working very hard now to modify the enzymes so that they will be taken up out of the bloodstream by the right cells."

Another problem Brady and his coworkers are making headway on is getting therapeutic enzymes through the blood-brain barrier — the lining surrounding blood vessels in the brain that keeps large chemicals from reaching brain tissue. They have managed, in rats and monkeys, to open the barrier for several hours at a time by injecting concentrated sugar solutions into the external carotid artery of the animals. The solutions open the barrier by shrinking, or drawing water from, the cells that comprise the barrier. The cells then separate from each other, and enzymes can slip through them into brain tissue. If the technique continues to look safe in animals, the NINDS researchers anticipate using it on patients with deficiencies of enzymes that are needed by the brain. Tay-Sachs victims, for instance, suffer severe mental retardation because of an inherited enzyme deficiency, and die several years after birth. Brady and his colleagues previously tried giving enzyme therapy to Tay-Sachs patients, but the enzymes could not get through their blood-brain barriers.

Yet another enzyme treatment that looks promising, but not for the immediate future, is being explored by Gerald Weissmann of New York University School of Medicine and his colleagues. Several years ago they planned to incorporate normal enzymes, which patients with Tay-Sachs disease, Niemann-Pick disease, the Hunter-Hurler syndrome and some other inherited enzyme diseases lack, into artificial membranes called liposomes, then to inject the liposomal-coated enzymes into patients with these diseases. By using liposomal-enshrouded enzymes, they reasoned, the patients' cells would not consider the enzymes as foreign and would let the enzymes through their own membranes. The researchers also anticipated that this treatment strategy could benefit rheumatoid arthritis and gout patients, who suffer a leak in critical enzymes rather than defects in enzymes.

In 1975 Weissmann and his team reported their first success along these lines. They managed to introduce the missing enzyme in the cells of patients with Tay-Sachs disease into white blood cells obtained from Tay-Sachs patients. These cells did not take up the free enzyme, but they did absorb enzyme encapsulated in liposomes (SN: 3/29/75, p. 211).

Getting liposomal-coated enzymes to work in animals and humans has been tougher, though. As Weissmann explains, "We're having trouble getting the liposomes into the bloodstream and directed to the cells that need them." To overcome these obstacles, the researchers are now tagging the liposomes with antibodies that should help direct the liposomal-packaged enzymnes to the appropriate target cells. Weissmann remains optimistic that liposomal-enzyme packets will eventually benefit persons with various defective or deficient enzymes.

Also on the horizon, and of keen promise to clinical medicine, is gene engineering of enzymes. Since the mid-1960s it has become possible to make a bacterium produce many copies of one enzyme; a cell usually contains 800 or 900 different enzymes. The technique works by inserting multiple copies of a gene for a particular enzyme into a circle of DNA called a plasmid, then slipping the plasmid into the bacterium's cytoplasm. The genes in the plasmid then make many copies of the enzyme in the bacterium's cytoplasm as the bacterium's nuclear DNA goes about its usual business of converting genes into various enzymes and proteins. (This gene engineering method is not quite the same as the much-publicized recombinant DNA technique in which a plasmid containing foreign DNA attaches to a host cell's DNA and may or may not integrate itself into the host cell DNA before making foreign proteins.)

Although gene engineering of enzymes is not yet benefiting clinical medicine, Pye foresees one way that it might. As mentioned earlier, people who are lactase deficient cannot tolerate milk because they don't have the enzyme lactase to break down the milk sugar lactose. But with gene engineering, as described above, bacteria could be made to produce lots of the enzyme lactase. The lactase enzymes could then be purified, attached to supports and used to break down lactose in milk into smaller sugars that are digestible by lactase-deficient people.

Obviously, now that techniques for exploiting enzymes medically are available, many more medical uses for enzymes will probably crop up in the near future. For as Pye explains, "Once you start people thinking, all sorts of wild things start happening. I think we are just at the tip of the iceberg right now." □