optimistic. "I see this new wave of telescopes making the NNTT more probable," says Arizona's Strittmatter. "The Palomar 5-meter in 1948 and the Lick 3-meter in the 1950's in fact triggered the development of the National Observatories, and the creation of instruments in places such as Mauna Kea and Chile. So, in the same way, I think these new instruments will make it clear just how exciting the opportunities are. I expect to see a real flowering over the next decade." ■ M. MITCHELL WALDROP

Making Antibodies Work Like Enzymes

The production of antibodies that can catalyze chemical reactions opens the way to making "enzymes" with any desired specificity

ANTIBODY proteins and enzyme proteins share a major point of similarity. They both bind their target molecules with high specificity and affinity. Over the years this resemblance has caused biochemists to wonder whether antibodies might also have the potential to behave like enzymes and catalyze chemical reactions. The answer is now in—and it is yes.

In this issue of *Science*, two independent groups of researchers, one at Scripps Clinic and Research Foundation in La Jolla and the other at the University of California at Berkeley, describe how carefully selected antibodies can catalyze the hydrolysis of certain organic compounds (pages 1566 and 1570). "The work shows for the first time that you can rationally design catalytic antibodies," says Peter Schultz of the Berkeley group. It opens up the possibility that the essentially unlimited diversity of antibody molecules can be tapped to produce enzymes with whatever specificities an investigator wants.

Catalytic antibodies might be designed, for example, that can cut proteins at any desired amino acid sequence. Researchers would like to have a battery of such enzymes that could be used for selectively dissecting proteins, much as restriction enzymes are used for dissecting DNA. The specificities of protein-cleaving enzymes are now largely limited to those provided by nature, however. The equivalent of restriction enzymes for proteins would be valuable for studying the relation between protein structures and function.

Catalytic antibodies also have potential medical applications. Antibodies by themselves do not destroy the target antigens, but essentially serve as signals for triggering the destructive activities of other immune system proteins and cells. Antibodies that can not only bind to proteins, but also cut them, might be useful for such applications as dissolving blood clots or searching out and destroying tumor cells.

The Scripps and the Berkeley groups approached the work with catalytic antibodies from different directions, but both depended on the same operating principle. Enzymes are generally thought to speed up chemical reactions by stabilizing the transition state, the most unstable and therefore the highest energy intermediate formed by the reactants during the conversion to products. Enzymes, by stabilizing the transition state, lower the energy needed for the conversion and consequently increase the rate of the reaction. The trick in producing catalytic antibodies lies in obtaining antibody molecules that will stabilize the transition states of the selected chemical reactions.

"We're excited about the work because this is a way of tapping into the vast repertoire of binding pockets [on antibodies] to do chemical work."

The Scripps workers, Alfonso Tramontano, Kim Janda, and Richard Lerner, began with a compound with a structure resembling the transition state of the reaction that they wanted to catalyze, which is an ester hydrolysis. They then used the compound as an antigen for generating monoclonal antibodies. The idea was that the antigen-binding sites of the antibodies produced would fit the transition state structure of the hydrolysis reaction. When such an antibody bound an appropriate chemical reactant, it would effectively stabilize that chemical in the transition state, thereby catalyzing the hydrolysis.

That is what happened. The Scripps group identified monoclonal antibodies that could speed up ester hydrolysis, although in the early work the catalytic antibodies did not behave exactly like true enzymes. One of the products of the reaction remained attached to the antibody molecule. A true enzyme releases the reaction products so that it can catalyze the reaction over and over again. In their more recent work, the Scripps group used different substrates for the catalytic antibodies, which they call "abzymes," and found that the products were released in the appropriate fashion.

The catalytic antibodies display a number of other characteristic enzyme features, in addition to speeding up the ester hydrolysis. For example, they show substrate specificity, hydrolyzing some esters but not others. The catalytic antibodies can be inhibited, as enzymes are, and the activity of the antibodies has at least a modest dependence on the *p*H of the reaction mixture. "We're excited about the work," Lerner says, "because this is a way of tapping into the vast repertoire of binding pockets [on antibodies] to do chemical work."

Schultz, with his Berkeley colleagues Scott Pollack and Jeffrey Jacobs, started their work with a preexisting antibody that binds the chemical nitrophenyl phosphorylcholine, which they realized is a transition state analog for the hydrolysis of structurally related carbonate compounds. The Berkeley workers found that this antibody catalyzes the hydrolysis of an appropriate carbonate in typical enzymelike fashion. They have since gone on to show that they can generate monoclonal antibodies that can also catalyze carbonate hydrolysis by immunizing with a transition state analog for the reaction.

The original antibody studied by Schultz, Pollack, and Jacobs belongs to a structurally well-characterized class of antibodies. The structural information available suggests, Schultz says, that the transition state in carbonate hydrolysis is stabilized by appropriately situated amino acid residues in the antigen-combining site of the antibody, just as predicted.

The catalytic antibodies identified by the Lerner group speed up the ester hydrolysis by a factor of about 1,000 and those under study at Berkeley accelerate the carbonate hydrolysis by a factor of perhaps 15,000. Nevertheless, as Lerner points out, the accel-

erations "are several orders of magnitude slower than what enzymes can do."

The relative slowness of the antibody-catalyzed reactions is not a major barrier to the eventual development and application of catalytic antibodies. Restriction enzymes do not work particularly fast either. Moreover, the work on catalytic antibodies is just beginning. Future investigations by the Schultz group, for example, will be directed at a better understanding of how the amino acids in the substrate binding site of the antibodies contribute to their catalytic activities. This information can help in the design of more effective "abzymes."

In addition, antibody genes are especially subject to mutations, which is one of the factors contributing to the generation of the large diversity of antigen-binding sites. Lerner suggests that it may be possible to apply genetic selection techniques to antibody-producing cells as a way of obtaining mutations that lead to the production of catalytic antibodies with the desired characteristics.

More chemical approaches might also be used to generate catalytic antibodies, Schultz points out. The Berkeley group is attempting to produce semisynthetic catalytic antibodies by chemical modification of the antigen-binding site. If this approach proves successful, the result would be a combination of the specificity and high binding affinity of an antibody with the activity of a synthetic catalytic compound not normally found in antibodies.

At least for now the major goal for the future is the production of catalytic antibodies that can break the peptide bond, which is the bond that joins together the amino acid building blocks of proteins. The catalytic requirements for breaking this bond will be more difficult to meet than those for ester or carbonate hydrolysis, however. "The problem is to get the appropriate chemistry into the binding pocket to carry out the more difficult reactions," Lerner says.

This situation is the converse of another current approach to making enzymes with particular specificities. Researchers are also trying to modify existing enzymes by using site-directed mutagenesis to change the amino acids in the substrate-binding sites, but in these circumstances they are usually trying to alter the range of substrates on which the enzyme will act, rather than the reaction catalyzed. With the antibodies, the specificity comes first. The trick then is to develop the catalytic activity. ■ JEAN L. MARX

ADDITIONAL READING

A. Tramontano, K. D. Janda, R. A. Lerner, "Chemical reactivity at an antibody binding site elicited by mechanistic design of a synthetic antigen," *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6736 (1986).

Math Proof Refuted During Berkeley Scrutiny

A highly publicized proof of a famous math problem—the Poincaré conjecture—has a gap, which might be unbridgeable

On Monday, 3 November, mathematician Colin Rourke of the University of Warwick got up in front of a cluster of mathematicians at the University of California at Berkeley to defend his claim that he and his colleague Eduardo Rego of the University of Oporto in Portugal had proved the Poincaré conjecture—a famous and difficult problem that has taunted mathematicians for 80 years. It was not an easy proof, and the mathematicians in attendance had already put in dozens of hours reading Rourke and Rego's work and trying to understand it. Now Rourke was about to start the first of several 3-hour seminars to explain the proof.

Because the Poincaré conjecture is such a famous problem and such a challenge to mathematicians and because Rourke and Rego had already gained a great deal of publicity for their proof, Rourke's seminars drew an impressive audience. In attendance were Berkeley's mathematical stars, including Andrew Casson and Robion Kirby. Well-known mathematicians from elsewhere came too, among them David Gabai and William Kazez of the California Institute of Technology. A few mathematicians who could not make it, including Fields Medal winner Michael Freedman of the University of California at San Diego, sent senior graduate students who served as emissaries.

But it was not to be the triumphant vindication that Rourke sought. By the end of the week, Rourke's audience pointed out what Rourke calls "a gap" that he cannot fill. Rourke says he is confident that he will be able to fix the proof, but others are not so sure. "My opinion is that what remains to be done is at least as difficult as what's been done already," says Casson. The opinion of the mathematicians at the seminars is that Rourke and Rego do not have a proof.

It is a familiar story in mathematics. The history of famous problems is littered with false proofs, some of them by eminent mathematicians who published proofs and only years later realized that they were incorrect. But what makes Rourke and Rego's proof stand out is the attention they received from nonmathematicians. Their work has been publicized in *Nature*, the *New Scientist*, and the *New York Times*, for example, at a time when the mathematics community was saying it remained to be convinced that the proof was real. The story of the decline and fall of this proof is more a story of the sociology of mathematics than of advances in math research.

The Poincaré conjecture was proposed at the turn of the century by French mathematician Henri Poincaré and it grabbed topologists' attention because, says Barry Mazur of Harvard, it is "so basic. If you are interested in geometry, the first thing you want to know are the simplest spaces." The Poincaré conjecture tells what they are.

The original conjecture applies to geometrical objects in three dimensions, but mathematicians generalized it to all dimensions. Freedman won his Fields Medal this year in part for his proof, in 1982, that the conjecture is true in four dimensions. In 1959, Stephen Smale of the University of California at Berkeley proved it is true for all dimensions higher than four. And it is fairly straightforward to prove it is true for dimensions one and two. So only the three-dimensional case remains unsolved.

The three-dimensional Poincaré conjecture is about the nature of the structures of three-dimensional objects, called three-manifolds. These are structures in four-dimensional space with the property that, says Kirby, "if you stand at one point and look around, it looks like ordinary three-dimensional space." An analogy is to a two-manifold, says Mazur. A doughnut and a balloon are two-manifolds. "If you are an ant on the surface of a doughnut and you look around, it looks like you are in a two-dimensional space," Mazur explains.

The Poincaré conjecture says that if you take a string, make a noose, and draw it closed on a three-manifold, and if the noose does not catch on anything as it is shrinking down to a point, then the three-manifold must be what topologists call a three-sphere—the four-dimensional analog of an ordinary sphere. In other words, only an object that is either a three-sphere or that can be stretched and pushed into a three-sphere has no holes that could snag the noose or leave it dangling.