for immunization. The vaccine now in use (killed *V. cholerae*) elicits formation of vibriocidal antibody, which reacts with the somatic, or cell-wall, antigen of the organism. In contrast, the antibody produced in response to pure toxin (or pure toxoid) is an antitoxin that inactivates toxin but has no vibriocidal activity. The usefulness of the killed vibrio vaccine is very limited because the immunity it evokes is not always sufficient to prevent cholera, and even when it is effective, the immunity lasts for only a few months or, at most, a year.

Finkelstein found that mice given crude cholera toxoid (either the natural toxoid or the toxin inactivated by formaldehyde) by either the subcutaneous or oral route developed antibodies to *V. cholerae* and were resistant to cholera when challenged with live bacteria. Because pathological changes in cholera are primarily in the intestine, Finkelstein has hypothesized that local antibody produced in the intestine may play a more important role in immunity to cholera than the antibody in serum. If this is correct, a vaccine that could be given orally rather than by injection might be advantageous because it would stimulate synthesis of intestinal antibody. Experiments done by William Burrows (*4*) and his co-workers at the University of Chicago support this idea. They found that intraintestinal administration of crude *V. cholerae* culture filtrates, which contain toxin and other bacterial products, elicited production of local antibodies to both the vibrios and the toxin.

In contrast, experiments done by the group at Johns Hopkins emphasize the importance of serum antibodies. These workers isolated an intestinal loop from a dog that had no antibody to *V. cholerae* and was, therefore, not immune to cholera; perfusion of this loop with blood from an immune dog (immunized by injection of crude toxin), which contained antibody, blocked the secretory response to cholera toxin. In the converse experiment, an intestinal loop of an immune dog was perfused with blood that lacked antibody to *V. cholerae*. In this case, the intestinal tissue was unable to prevent the action of cholera toxin.

Recent work indicates that both serum antibody and intestinal antibody are important. Robert S. Northrup of the National Institutes of Health (NIH) and Pierce found that dogs immunized by subcutaneous or intramuscular injection of cholera toxin and adjuvant (a substance that enhances the immune response) were resistant to challenge with *V. cholerae* for 10 months and had antibodies in their serums for 18 months (*5*). Moreover, when cholera toxoid was used for immunization, dogs retained resistance to *V. cholerae* for 9 months. After this time, toxoid given orally as a booster immunization provided renewed protection although titers of antitoxin in the serum did not increase. This last finding suggests that the protection conferred by antitoxin is partly due to secretory antibody in the intestine. Furthermore, the protection could be attributed almost solely to antibodies to the toxin; the dogs had only very slightly increased amounts of vibriocidal antibody.

*Speaking of Science*

# Chemistry in 1972:

Nineteen seventy-two was not a banner year for science. Even though the year was marked by the final landing on the moon and the launch of the "great crusade against cancer," there didn't seem to be any events with the memorable impact of a first synthesis of an enzyme, a new Salk vaccine, a new thrust into space, or a first heart transplant. It was, rather, a year of slow but steady advances, the inch forward instead of the great leap.

The American Chemical Society (ACS) has just published a short booklet (*1*) reviewing the year and highlighting the broad spectrum of chemical developments, both scientific and technological. The booklet, prepared under the auspices of the society's Professional Enhancement Program to improve employment opportunities for chemists, is perhaps somewhat self-serving and tends to be biased toward developments with commercial significance. Nonetheless, it provides a small measure of man's growing comprehension of the world around and within him.

Among the highlights of 1972 selected by ACS:

► The first elucidation of the chemical structure of a gene, by Walter Fiers at the State University of Ghent, Belgium. Fiers' group determined the complete nucleotide sequence for the 387-unit RNA strand that governs synthesis of the protein coat of bacteriophage MS2, a small virus, and deduced the "flower-like" geometric configuration of the backbone of the molecule. Comparison of the known amino acid sequence of the coat protein with the newly determined gene structure also reaffirmed the identities of the three-unit codons that form the genetic code.

► Strong support for the theory of convergent evolution from Joseph Kraut of the University of California at San Diego. By x-ray crystallography, he showed that two types of enzymes which catalyze the hydrolysis of polypeptides—subtilisin from bacteria and molds and chymotrypsins from bacteria and animals, including man—embody essentially the same catalytic groups and binding apparatus, despite gross structural differences. He thus presumes that the two enzymes evolved convergently from different ancestral enzymes toward the same biochemical function.

► The commercial introduction of prostaglandins for routine, nonexperimental medical use. Prostaglandins, 20-carbon fatty acids, have been implicated in a wide variety of metabolic control mechanisms, frequently as a mediator between hormones and cyclic adenosine monophosphate, and are assumed to be the most promising major class of drugs since sulfanilamides. In the autumn of 1972, the United Kingdom approved two prostaglandins made by the Upjohn Company, Kalamazoo, Michigan, for use in inducing labor and in terminating pregnancy. These prostaglandins are thought to act by inducing contractions of the uterus and by inhibiting progesterone secretion in the ovary; progesterone normally must be present for the fertilized egg to be implanted in the uterus.

► New evidence that man's production of carbon monoxide is dwarfed by nature's, from C. M. Stevens of

Although the toxin is an effective vaccine in animals, it is unsatisfactory for use in man because it induces swelling at the site of injection. Toxoid is immunogenic yet does not produce adverse reactions. Therefore, a major aim of the NIH Cholera Advisory Committee is the development of a toxoid vaccine suitable for use in man. According to Northrup, the first step is to prove that it is the toxoid itself, and not an impurity, that is the active immunogen. Impure toxoid contains some of the cell wall antigen of *V. cholerae*; therefore, resistance to cholera induced by impure toxoid could be due, in part, to vibriocidal antibody.

Since the highly purified toxoid prepared by treatment of toxin with formaldehyde reverts to active toxin, crude preparations (which do not revert) were used in most experimental vaccinations of animals with toxoid. Recently, Wyeth laboratories prepared a pure toxoid by treatment of toxin with glutaraldehyde rather than formaldehyde; toxoid prepared in this manner does not revert to toxin, and preliminary experiments indicate that it is a safe and effective vaccine in rabbits and monkeys.

Large-scale field trials of the toxoid vaccine may be forthcoming. Work safety, antigenicity, and correct dosage of the glutaraldehyde-treated toxoid in human volunteers is now in progress. Later, volunteers vaccinated with the toxoid will be challenged with live *V. cholerae*. If these studies with volunteers yield convincing evidence that toxoid protects against disease, toxoid vaccine will be given to a large population in rural Bangladesh, possibly by 1974. The incidence of cholera among this population will be compared to the incidence in a similar but unvaccinated population. A significantly lower rate of cholera among the vaccinated group would show the efficacy of the vaccine and warrant further trials of the toxoid as a vaccine, used both alone and in combination with the older, killed vibrio vaccine.

Because the killed vibrio vaccine does confer some (although inadequate) protection against cholera, there is reason to expect that a vaccine containing both somatic antigen of *V. cholerae* and toxoid might be superior to a vaccine consisting of one or the other alone. Thus it is quite possible that the ultimate vaccine will be an impure preparation of toxoid which contains both antigens. Such a vaccine would not be difficult to make on a large scale. If immunity can be maintained in man, as it was in dogs, by oral boosters given periodically after initial vaccination by injection, it should be feasible to vaccinate and protect large numbers of people against cholera in areas of the world where the disease is either endemic or epidemic.

—MARY M. EICHHORN ADAMS

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# Not a Leap, but an Inch

Argonne National Laboratory, Argonne, Illinois. By measuring the carbon and oxygen isotopic ratios in atmospheric carbon monoxide and thus determining its origins, Stevens found that natural sources produce some 3.5 billion tons of carbon monoxide annually in the Northern Hemisphere, primarily by atmospheric oxidation of methane from decaying vegetable matter. Since man produces only about 270 million tons, the finding puts to rest the theory that man is disturbing the atmospheric carbon monoxide balance.

► Commercial introduction of immobilized or bound enzymes. Soluble (unbound and, hence, unrecoverable) enzymes have been used for many years, mainly in food processing, but these enzymes must necessarily be relatively inexpensive because they can be used only once. By attaching the enzyme to an insoluble support, it can be recovered and used many times, thus greatly increasing the number of enzymes whose use is economically feasible. Clinton Corn Processing Company, Clinton, Iowa, and CPC International Inc., Englewood Cliffs, New Jersey, have developed bound enzyme processes for isomerization of dextrose to fructose, thus making "invert sugar" available from cornstarch as well as the normal source, sucrose. Corning Glass Works, Corning, New York, in 1972 began selling glass beads with a silane coating to which enzymes can readily be bound, and will soon sell the beads with commonly used enzymes already attached.

► Simulated enzymic synthesis of sterols by Eugene van Tamelen of Stanford University, Stanford, California. After more than a decade of effort, van Tamelen developed a nonenzymic cyclization of a substituted squalene oxide to dihydrolanosterol, a biologically important sterol, that virtually duplicates the enzymic synthesis. The work led to the development of broadly applicable routes for synthesis of large cyclic molecules, and may provide fundamental information about how the enzyme functions.

Other major developments cited by ACS include the detection of a rudimentary "memory" in bacteria, by R. M. McNab and D. E. Koshland, Jr., of the University of California, Berkeley; the identification on the Orgueil meteorite (which fell in France in 1864) of six amino acids not commonly found in protein, suggesting that they originated somewhere other than the earth, by Cyril Ponnamperuma, now at the University of Maryland, College Park; and the identification and synthesis of two sex attractants from the Mediterranean fruit fly by Martin Jacobson of the U.S. Department of Agriculture, Beltsville, Maryland.

Disparate as these developments may seem, they nonetheless share one leitmotif: they represent not so much single bursts of discovery, but rather persistent, often multidisciplinary extensions of research that has gone before. By the same token, current chemical progress may perhaps foreshadow achievements unthought of in the year just ended.—THOMAS H. MAUGH II

**References**

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