will be launched later this year, to be followed by a Mariner Jupiter-Saturn mission in 1977. The Viking orbiter/lander mission to Mars is set for 1975–76. Other activities will include the launching of the Orbiting Solar Observatory in 1974, of two German-American solar probes in 1974 and 1976, and of a number of technological "applications" satellites (for earth resources reconnaissance, weather studies, and the like) between now and the end of 1977. With the foregoing manned and unmanned space activities, together with a modest program in aeronautics, NASA would have about 25,000 civil service employees throughout the 1970's and support about 100,000 contractor personnel (the latter figure going somewhat higher at the peak of work on the space shuttle).

A clear indication that NASA's major programs were safe (certainly for the moment) came several weeks ago when the agency, faced with White House demands to do its part toward holding total federal spending for fiscal 1973 to a $250-billion ceiling, escaped with a cut of only $179 million. To make the cut, development of the shuttle was ordered slowed by somewhat less than a year off of its original schedule and the launch dates for two of the technological applications satellites was ordered delayed. In addition, there were decisions to suspend the High Energy Astronomy Observatory project (pending redesign of HEAO in a cheaper configuration), to phase out the communications satellites project (letting industry take over), and to terminate long-term projects for development of nuclear propulsion and large-scale nuclear power sources.

Should there ever come a decision to kill or indefinitely postpone the space shuttle, the agency's status may slip to that of an inconspicuous scientific and technological agency quietly doing interesting but not very exciting things. The shuttle is in fact critical to NASA's future, as that future is now envisioned. During this decade as much as a third of the agency's civil service personnel and up to one half or more of its contractor personnel will at times be working on this project. And, for the long term, once the shuttle becomes operational—at a total cost of at least $6.5 billion—an ambitious program of flights will have to be carried out to justify having built it. In terms of cost-effectiveness, the shuttle does not start breaking even unless at least 30 heavy scientific, military, or other payloads are launched annually over a 12-year period.

NASA officials probably are not going to be able to rest easy about the shuttle until a few billion dollars have been spent on it. Not more than about $775 million will have been spent by the close of fiscal 1974—little enough that the Administration might be tempted to cancel the project should severe budgetary difficulties again arise.

Yet NASA officials seem confident that the shuttle will be built, and there perhaps is little reason to believe otherwise. President Nixon has supported the project—although his new budget message contained no mention of the space program whatever—and, in Congress, it has survived handily all past attempts to kill it. The fact that the project helps sustain an aerospace industry that has suffered grievously from layoffs is a point lost on no one. And, then too, NASA has going for it the fact that, both in Apollo and in the unmanned programs, it has generally met its goals and stayed within its budget.—L.J.C.

Supersonic Technology

Ever since that day two years ago when the White House lost, by a close vote in the Senate, the battle to keep the supersonic transport alive, there has been speculation that President Nixon would ultimately seek to revive the project. The evidence now is that the President does indeed look to a possible revival of the SST, but not until later in the 1970's. The new NASA budget contains $28 million—more than twice as much as last year's budget—for research and development on supersonic technology. The work will focus on problems of noise, pollution, and efficiency of configuration.—L.J.C.

RESEARCH NEWS

Cholera: New Aids in Treatment and Prevention

The current epidemic of cholera, which began a decade ago in Indonesia and is still rampant on the Indian subcontinent and in Africa, has stimulated research on the biochemical and immunological, as well as the clinical, aspects of the disease. It is now known that the profuse diarrhea of cholera is caused by a toxin made by *Vibrio cholerae*, the bacterium that causes cholera. In the intestine this toxin stimulates the enzyme adenylate cyclase, which in turn causes production of an excessive amount of cyclic adenosine 3′,5′-monophosphate (cyclic AMP). Then, by a mechanism still not understood, the cyclic AMP induces the hypersecretion of water and salt characteristic of the disease.

Effective therapy for cholera now consists of replacement of the water and salt lost through diarrhea; when initiated early enough, this therapy can save almost all victims. Although cholera is best prevented by modern sanitation and clean water supplies, improved vaccines may be a more readily attainable means of reducing the incidence of cholera in underdeveloped areas of the world. Preliminary evidence suggests that an inactivated form of the toxin (toxoid) may be superior to the vaccine made of killed *V. cholerae* that has long been in use.

The diarrhea caused by cholera results in the loss of large amounts of body fluids and electrolytes (sodium, potassium, chloride, and bicarbonate ions). Although these losses have been attributed to the effects of cholera toxin, the precise site of action of the toxin was identified only recently. For example, Michael Field, working first at Johns Hopkins University and later at the Beth Israel Hospital in Boston, discovered that cholera toxin increased the amount of chloride ion secreted by the intestine and decreased the net ab-

sorption of sodium by that tissue. Experiments performed in a segment of intestine isolated by ligation from intact, live rabbits showed that cyclic AMP produced exactly the same changes in ion transport across the intestine as cholera enterotoxin did. The altered patterns of transport of sodium and chloride were linked to an increase in the potential difference and in the short-circuit current across the intestinal wall. Further evidence for common mediation of the effects of cyclic AMP and cholera toxin was provided by an experiment showing that the pattern of ion transport by the intestine, once altered by enterotoxin, was unaffected by cyclic AMP.

The implication that cyclic AMP was involved in the changes wrought by cholera toxin was strengthened by the work of Nathaniel F. Pierce, W. B. Greenough III, and Charles C. J. Carpenter (see *1*) at Johns Hopkins University and by Field who found that prostaglandins, known to be activators of the enzyme adenylate cyclase in some cells, mimic the effects of cholera enterotoxin. When prostaglandins were infused into the arteries that supply blood to the intestines of dogs, the pattern of secretion of fluid and ions by an isolated segment of intestine was identical to that produced by cholera toxin. Further experiments demonstrated that the toxin affected processes mediated by cyclic AMP in other tissues as well. Hence investigators concluded that the activity of cholera toxin is not specific for the intestine. However, unlike prostaglandins, whose effect is reversible, cholera toxin permanently affects the adenylate cyclase of cells.

After David E. Schafer, working at the University of Minnesota, reported that cholera toxin caused an increase in the concentration of cyclic AMP in the intestine, the idea that the toxin acts by stimulating adenylate cyclase was confirmed by Geoffrey W. G. Sharp and his associates at Harvard Medical School and the Massachusetts General Hospital, who worked in collaboration with Lincoln Chen at the Cholera Research Laboratory in Dacca, East Pakistan (now Bangladesh) (*2*). Sharp and Chen discovered that the activity of the enzyme was more than twice as high in intestinal biopsy specimens from patients with active cases of cholera as in specimens from patients convalescing from the disease. Studies on adenylate cyclase in cell-free preparations showed that the enzyme in tissues from patients with cholera has the same properties, including stimulation by prostaglandins, as enzyme from normal tissue. These results appear to rule out the possibility that cholera toxin causes synthesis of an altered molecular form of adenylate cyclase.

In another series of experiments, the transport of sodium and water across isolated loops of canine bowel and the activity of adenylate cyclase in the tissue were measured simultaneously; the results showed that enzyme activity paralleled the net flux of water and sodium. The changes induced by the toxin were not immediate, but the difference from controls was significant after 1 hour; both activity of the enzyme and flux of sodium and water returned to normal after 48 hours. These observations are considered to be firm support for the hypothesis that loss of water and electrodes through the intestine during cholera is mediated by adenylate cyclase and cyclic AMP.

Adenylate cyclase is not the only enzyme affected by cholera toxin. Sharp and his co-workers have found that the activity of sodium and potassium dependent adenosine triphosphatase of intestinal cell membranes is reduced as much as 60 percent by the toxin. The physiological significance of this finding is not yet understood; however, since both adenylate cyclase and adenosine triphosphatase are membrane-bound enzymes, it has been proposed that phospholipids in the cell membrane may be involved in the effect of cholera toxin on the two enzymes.

Therapy

Understanding of the biochemical basis for the action of cholera toxin was preceded by the development of effective therapy by Norbert Hirschhorn and his colleagues at the Cholera Research Laboratory in Dacca and by Pierce's group, then in Calcutta. These groups found that the water and salt lost from the body can be replaced if the patient drinks a solution containing glucose, sodium chloride, potassium chloride, and sodium bicarbonate. Glucose promotes intestinal absorption, thereby reducing the loss of water and sodium. Thus, glucose is a key ingredient of the orally administered solution. This treatment has permitted extension of medical care to many more victims of cholera than could be treated previously by intravenous replacement of fluid and salt, a method requiring medical personnel and facilities often unavailable where cholera is endemic. Additional advantages of oral therapy are low cost and simplicity; it can be given by a patient's relatives with little or no medical supervision. In severe cases, oral therapy is supplemented by initial intravenous therapy; nearly all patients receive the antibiotic tetracycline, which kills *V. cholerae*.

Although replacement of fluid and salts and administration of antibiotics constitute the only therapy now in use, several pharmacologic approaches to the treatment of cholera have been proposed. Sharp has suggested two mechanisms that would lower the concentration of cyclic AMP in the intestine: stimulation of the enzyme phosphodiesterase (which destroys cyclic AMP) or inhibition of adenylate cyclase. Simple sugars, such as erythrose and glyceraldehyde, inhibit adenylate cyclase in vitro, but the effects of these agents on the enzyme have not been fully tested in vivo.

Another therapeutic possibility lies in the development of drugs that block the binding of cholera toxin to intestinal cells. Richard A. Finkelstein at the University of Texas Medical School in Dallas found, by immunohistochemical methods and autoradiography of tissue from mice exposed to toxin, that the toxin binds specifically to the membranes of the villi (*3*). This binding is so rapid that antitoxin, given only several minutes after toxin, fails to decrease loss of fluid. A naturally occurring toxoid found in culture filtrates of *V. cholerae* also binds to the cells, but does not cause movement of fluid. This toxoid does, however, prevent the action of toxin. In addition, gangliosides, complex molecules of lipid and sugars present in cell membranes, bind to the toxin and block its effect both in isolated intestinal loops of animals and in fat cells whose lipolytic activity was studied in vitro. Rapid binding of cholera toxin to the intestinal cells is evident from the observation that, to block the action of toxin, ganglioside must be added within 15 minutes after addition of toxin.

Toxin-Induced Immunity

The experiments that led to the elucidation of the mechanism of action of cholera toxin depended on use of a pure preparation of cholera toxin. After many years of work, Finkelstein's group succeeded in isolating the toxin in pure, crystalline form. Since the toxin is a protein and antigenic, recent efforts in the field of cholera immunology have focused on the potential use of an inactivated form of cholera toxin

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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References

1. C. C. J. Carpenter, *J. Infect. Dis.*, in press.
2. L. C. Chen, J. E. Rohde, G. W. G. Sharp, *J. Clin. Invest.* **51**, 731 (1972).
3. J. W. Peterson, J. J. LoSpalluto, R. A. Finkelstein, *J. Infect. Dis.* **126**, 617 (1972).
4. J. Kaur, W. Burrows, M. A. Furlong, *ibid.* **124**, 359 (1971).
5. N. F. Pierce, E. A. Kaniecki, R. S. Northrup, *ibid.* **126**, 606 (1972).

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

1. *Chemistry in 1972* (American Chemical Soc., Washington, D.C., 1973).