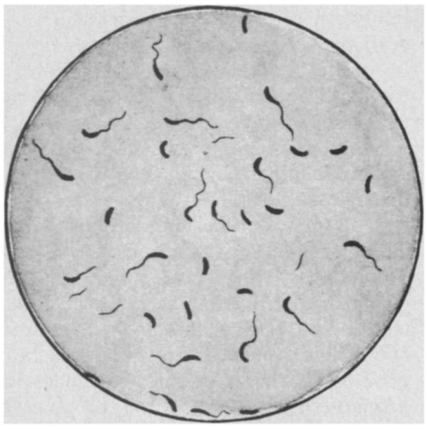
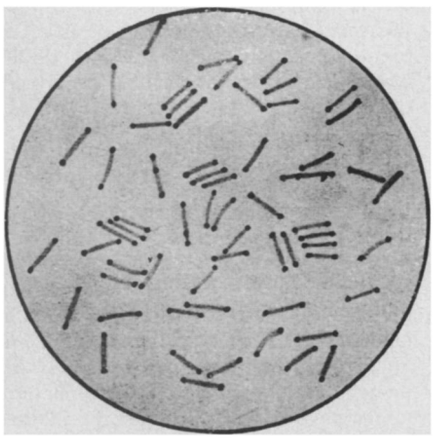
How Bacterial Toxins Can Do You In

Scientists are scrutinizing the chemistry of these, the world's most virulent biological substances, and how they poison at the tissue, cell and even molecular level

BY JOAN AREHART-TREICHEL



Vibrio cholerae (× 1,200)



Corynebacterium diphtheriae (× 1,000)

Nov. 27, 1974, was like any other day for Gladys G. and Emily R., two widows living next door to each other. They had a leisurely lunch together. That evening, each ate a simple meal in her own home. The next day, each went to her son's home for Thanksgiving dinner. What they didn't know was that the food they ate during these two days was going to change the course of their lives.

Immediately after Thanksgiving dinner, Gladys started to vomit. The next two days she complained of a sore throat, and her voice changed. Emily experienced similar symptoms. On Dec. 1, both women were hospitalized. By now their facial, tongue and arm muscles were incredibly weak. The hospital staff drew blood samples from them and examined the samples for toxins. Sure enough, they found what they feared: the botulinus toxin. By the time the hospital had discovered the source of their poisoning—stew they had shared for lunch—Gladys died. Antiserum to the toxin helped save Emily's life.

It's hard to believe that such grave and lethal consequences of poisoning could have been caused by a tiny organism that cannot be seen without a microscope—the bacterium *Clostridium botulinum.* Just as incredible, still other bacteria make toxins that can inflict serious injury on people and even threaten their lives. There's *Clostridium tetani,* which causes tetanus; *Corynebacterium diphtheriae,* which causes diphtheria; *Vibrio cholerae,* which triggers cholera; *Pseudomonas aeruginosa* and other gram-negative bacteria that can bring about shock and even death if they get into the bloodstream. Although *Staphylococcus aureus* and *Clostridium perfringens* make toxins that are rarely fatal, the toxins do induce vomiting and severe diarrhea.

These toxins constitute the world's most virulent biological poisons. The botulinus toxin is the most potent of all. One ounce would be enough to kill 60 million people. Needless to say, scientists are eager to learn more about the chemical composition of these toxins and how they damage the body. They are making strides in both directions. Nonetheless, questions about the chemistry and actions of the toxins are still pressing for answers.

The botulinus, tetanus, diphtheria and staphylococcus toxins appear to be medium-sized proteins, with weights from about 20,000 to 150,000 daltons, scientists have found in recent years. However, it has been difficult to determine their amino acid sequences, at least those of the larger ones. For instance, attempts have been made to determine the amino acid sequence of the botulinus toxin. But the problem, as E.J. Schantz, a bacterial toxin chemist at the University of Wisconsin, points out, "is to get a good pure, low-molecular-weight fraction. Some scientists have tried it, but it has not been complete."

Even without unraveling the amino acid sequences of these toxins, though, investigators are obtaining an idea of which parts are critical for toxicity. In recent years, for example, they have found that the botulinus toxin fluoresces. This feat in itself is not remarkable. All proteins fluoresce because of the presence of three aromatic amino acids—tryptophan, tyrosine and phenylalanine. What appeared to be remarkable, however, is that whenever fluorescence was destroyed, the toxin's virulence was too. This discovery and some others led the late D.S. Boroff and his colleages at the Albert Einstein Medical Center in Philadelphia to suspect that tryptophan might be one of the critical amino acids behind the toxin's potency. They knocked tryptophan out of the toxin. Sure enough, it was no longer poisonous.

In the opinion of D. Michael Gill of Harvard University, however, such sleight-of-hand efforts don't really illuminate which amino acids in the molecule are crucial for toxicity. "Obviously, if a protein has a tryptophan in it," he says, "and you start to monkey around with it, you are very likely to interfere with its biological activity. You can do that with hundreds of proteins, as far as I know. It really doesn't help at all in understanding how a toxin works to say, 'Well, if you modify this particular amino acid, the toxicity is destroyed.'"

Still other evidence suggests that toxins' virulence depends more on their three-dimensional than on their two-dimensional structures. Schantz and his colleagues have found that if they treat botulinus toxin with urea, a reagent that distorts the shape of the molecule, the toxicity is destroyed without a change in the molecule's fluorescence.

As for the gram-negative bacterial toxins, a number have been found to be lipopolysaccharides (fats hooked to sugars). The chemical nature of these toxins is now reasonably well understood. The uncertainty rests on which portion or portions are essential for toxicity.

So, courtesy of their molecular quirks and eccentricities, bacterial toxins can inflict various kinds of damage to the body. Botulinus toxin, for instance, leads to vomiting within 12 to 36 hours after consumption. Weakness then develops, followed by paralysis. Death occurs from inability to breathe. The reason that the toxin is able to produce these effects, investigators have found, is because the toxin attacks the nerve-muscle junction. Specifically, it inhibits the release of the nerve transmitter acetylcholine. Muscles are paralyzed as a result, particularly those responsible for breathing.

Tetanus toxin, on the other hand, acts just the opposite from botulinus toxin. It triggers spasms of muscles, particularly of the jaws (hence the name "lockjaw"). The toxin causes these spasms, scientists

have learned, by preventing the normal inhibition of nerve synapses. The toxin probably enters the central nervous system by way of peripheral motor nerves. Once the toxin binds to nerve synapses, it can no longer be reversed therapeutically.

Diphtheria toxin is not nerve-specific as are the botulinus and tetanus toxins. It can damage many organs, notably the heart, leading to sudden heart failure and death. One of the reasons that cells are susceptible to the toxin, Frank Ruddle and his team at Yale University have found, is due to the presence of chromosome number five. They suspected that there may be a gene on this chromosome that codes for a diphtheria toxin membrane receptor. Or the gene may make an enzyme that modifies the cell's membrane in such a way that it will bind to the toxin. After diphtheria toxin gets into the cell, it then interferes with protein synthesis. The toxin specifically inactivates one of the elongation factors involved in the growth of the polypeptide chain.

Pseudomonas toxin, investigators have found, goes for liver, kidney and spleen cells, then inhibits their protein synthesis. How the toxin inhibits protein synthesis was not known until recently. Then Barbara H. Iglewski and David Kabat of the University of Oregon Medical School found that the toxin results in a block at an elongation step of polypeptide assembly, just as diphtheria toxin does. "Although pseudomonas and diphtheria toxins have different cellular specificities and molecular properties and produce different clinical symptoms, their intracellular mechanisms appear to be identical," they conclude.

If pseudomonas toxin or any other gram-negative bacterial toxin gets into the bloodstream, it decreases the number of platelets. The reason is that platelets, traitorously, have specific receptors for the toxins on their membranes, Jacek J. Hawiger, and his colleagues at Vanderbilt University School of Medicine recently found. Such a discovery, they stress, is important because "platelets are intimately involved in activation of blood coagulation, and their interaction with toxin may help us to understand better toxin-induced intravascular coagulation and shock."

As for cholera toxin, it triggers extensive diarrhea, vomiting, muscle cramps and collapse and will lead to death if not treated. Complications such as pneumonia and serious skin infections can delay recovery. The action of cholera toxin in the body has been extensively explored. Scientists now know that many types of cells are sensitive to cholera toxin, and that the toxin first interacts with a limited number of receptors on their membranes. The toxin then provokes the activation of the intracellular messenger cyclic AMP.

But how does the toxin do this? The toxin first acts on the membrane enzyme, adenylate cyclase. A component in the cell's cytoplasm, nicotinamide adenine dinucleotide (NAD) then serves as an abettor, Gill and some other researchers have found. However, Naji Sahyoun and Pedro Cuagrecasas of Johns Hopkins University School of Medicine report just the opposite—that components in the cytoplasm do not seem to be necessary for cyclic AMP activation.

In any event, stimulation of cyclic AMP appears to be the key to the toxin's cellular action. What's more, cyclic AMP activation can help explain why the toxin leads to such extensive diarrhea, Jan Holmgren and his team at the University of Goteborg, Sweden, and other toxin researchers concur. Overproduction of cyclic AMP could lead to the oversecretion of chloride from cells, and the chloride could trigger diarrhea.

Even though toxin investigators now know a lot about bacterial toxin chemistry and actions, they are not about to lock up their labs. They want to learn more about the compositions of these toxins and the parts that are responsible for toxicity. Regarding gram-negative bacterial toxins, for instance, one current notion is that the lipid portion is the culprit. Researchers also want to learn more about the actions of bacterial toxins in the human body. Schantz, for example, is intrigued by the discovery that the botulinus molecule starts off as a protein with a molecular weight of 900,000, then splits into a 150,000-weight toxin and a 750,000-weight nontoxic protein. "It seems as if the big molecule stabilizes the toxic part," he speculates. "If you didn't have the whole molecule, you would probably not get poisoned, because it would not be stable enough to survive the digestive tract and be carried into the blood and on to the site of action."

Bacterial toxin behavior at the molecular level is still another area that researchers want to plumb further. As Iglewski and Kabat point out, substantial information at this level is known for only three of the toxins—diphtheria, pseudomonas and cholera. And even with these three, scientists are eager to probe the miasma further. Gill, for instance, wants to understand NAD's precise role in helping cholera toxin activate cyclic AMP. "Only then," he insists, "will we have a full understanding of cholera."

Researchers would also like to know exactly how much the bacterial toxins resemble each other in their actions and why. As Iglewski and Kabat point out, "We were surprised to find that both diphtheria and pseudomonas toxins have the same intracellular mechanism of action. The probability of this happening by chance or convergent evolution is obviously remote. Accordingly, we suggest that these two toxins may have had a common evolutionary origin and that some other bacterial toxins will be found to act similarly."

Probably the question that still nettles toxin researchers the most is why bacterial toxins are the world's most virulent biological substances. "We don't know the answer, and we'd sure like to," Schantz admits. The answer will undoubtedly only come to light as researchers unravel the two- and three-dimensional structures of these molecules and thoroughly unmask their baleful actions both inside and outside various cells. □

. . . Trains

erable export potential. Newly rich countries of the developing world are already entering the market for advanced rail equipment. Iran has just become the third country in the world to schedule trains over 100 miles per hour, by placing two French Turbotrains on the line from Teheran to Mashhad. But the largest potential market must still be considered the United States. Amtrak's timetable is about one quarter the size of the Scottish regional timetable of British Rail. Though the total length of track used for its passenger service is only one quarter that of the United States, British Rail Intercity Line logs twice as many passenger miles per year as Amtrak, and more than six times the passenger journeys (neither figure includes commuters). Already the U.S. Department of Transportation has given contracts to the British Rail Research and Development Division for consultation (including contributions to a research vehicle that set a 234-mile-per-hour world record in Colorado). If President Ford's commitment to revitalizing America's railways as a part of energy conservation begins to bear fruit (SN: 1/24/76, p. 52), some of the innovations pioneered at Derby may find a ready market here.

A final note of caution, however, must be added. Even as this article was in preparation, the British Government was preparing a position paper that some press accounts predict will close nearly a third of Britain's 11,500-mile passenger rail system. Rail officials will welcome some of the closures (one of them told SCIENCE NEWS it would be cheaper to buy cars for some remote villagers than maintain train service), but any shift of emphasis to trucks as a freight medium would come as a shock. As in this country, the intrinsic economies of railways versus roadways are obscured by complex labor and social issues, and whether a nation as deeply into recession as the United Kingdom will provide the tens of millions of pounds needed to complete the APT remains to be seen.

Technologically, however, that venerable old British invention, the Iron Horse, is still up there with the best of them. □