# Collaboration and Complex Techniques Led to Discovery of Curare in Tissues

NYTimes MAR 8 1976

By LAWRENCE K. ALTMAN

The identification of curare in a patient involved in the Dr. X case resulted from an extraordinary collaborative effort by doctors and scientists in New Jersey and New York who tested specimens from bodies that had been exhumed 10 years after death—one of the longest intervals known to pathologists.

On the basis of the curare identification that was disclosed yesterday, the Dr. X case seems likely to become one of the most widely discussed episodes in the annals of medicine. And it is an episode so bizarre as to rival the imagination of Sir Arthur Conan Doyle, himself trained as a physician.

When the medical detectives in the Dr. X case began their work, nothing in their own experience or in the medical literature told them if curare could be detected in decomposed bodies so long after it was injected. Neither did the investigators know if the bodies would have been sufficiently well-preserved to make the effort worthwhile.

But over the last two months, pathologists, toxicologists, dentists, anesthesiologists and immunologists in the two states have used a variety of techniques, ranging from the most basic—fingerprints, dental impressions and x-rays—to the most advanced, including some that had not been fully developed or commonly available at the time the patients died a decade ago.

These investigators relied on tissues obtained at the exhumations of five patients who died suddenly and mysteriously at Riverdell Hospital in Oradell, N.J., to check on the accuracy of the originally cited causes of death and to determine if there was any validity to the suspicions raised by the Bergen County Prosecutor about curare, which can be deadly.

Curare has been found in the tissues of the first body exhumed and appears to be present in other bodies as well, but the tests on all exhumed bodies have not been completed, according to law enforcement sources.

Samples of liver, kidney, lung and other biologic tissues were ground-up for an array of tests. Technicians sprayed coated glass plates with dyes to detect violet spots indicating the presence of curare. They looked under ultraviolet lights for fluorescent reactions, passed specimens through jets of gas, used "laboratory handguns" to precisely squirt into test tubes minute quantities of liquids, and then analyzed data

Continued on Page 43, Column 1



Continued From Page 1, Col. 7

spewed forth by computers that measured test results in a trillionth of a gram.

Toxicologists from the New Jersey and Suffolk County Medical Examiner Offices in Newark and Smithtown, L.I., collected specimens while Dr. Michael M. Baden, New York City's Deputy Chief Medical Examiner, autopsied the exhumed bodies.

The specimens were divided so that the toxicologists could apply as many independent physical and chemical methods as possible to detect curare. Further, if curare was detected, independent confirmation by two teams would strengthen the validity of the scientific findings. The results were vital not only to the Dr. X case but also to future exhumations in which technology and knowledge gained after burial might help determine the cause of death at a later date.

### Team Assembled

Dr. Baden, acting on a New Jersey Superior Court order, assembled a team of investigators from medical schools, commercial laboratories and two other medical examiner offices, to conduct the analysis.

In addition to Dr. Baden, the group was: Dr. Donald Hoffman and Dr. Lorenzo Galante at the Medical Examiner's Office here, Dr. Edwin H. Albano and Dr. Richard Coombis at the New Jersey Medical Examiner's Office, Dr. Sidney B. Weinberg and Dr. Leo A. Dal Cortivo at the Suffolk County Medical Examiner's Office, Dr. Richard S. Matteo at Columbia College of Physicians and Surgeons. Dr. Robert E. Finnigan at Finnigan Corporation in Sunnyvale, near San José, Calif., is also involved in the testing of tissue.

In separate interviews, members of the team described the scientific steps they took to seek identification of curare. Though they discussed the methodology, none of the investigators would discuss the results, which were disclosed by other sources.

Curare had not been prescribed for any patients involved in the Dr. X case. The drug came under suspicion because of the pattern of repeated sudden respiratory deaths among post-operative patients at Riverdell and because many empty vials of curare were found in Dr. X's locker.

Curare—called "flying death" because Indians living in South American jungles and forests traditionally dipped the tips of their arrows in the poison is actually a generic term to cover several poisons obtained from a variety of plants. It is used against enemies and to kill animals for food.

As curare's reputation spread as one of the deadliest poisons known, doctors shied away from experimenting with it on humans. Not until just before World War II was curare introduced into medicine, for treatment of the muscle spasms of tetanus and to help prevent fractures of the spine in electroshock therapy.

Anesthesiologists also found that by injecting curare and then giving artificial respiration, they could drastically lower the dose of anesthetic gases to many surgical patients. These had proved dangerous in high amounts for prolonged periods.

The discovery of a safe use for curare had a revolutionary impact on medicine, particularly in surgery, by making operations much safer. In the initial period of its use, however, anesthesiologists ascribed the deaths of some patients to inadvertent overdoses of the curare in the operating room.

### Paralysis Can Occur

Though curare and similar drugs later marketed are now used in hospitals throughout the world, doctors say they still would like to know more about the physiology of how curare blocks nerve impulses at the junction of nerve and muscle cells. Dispersion of about 30 milligrams of curare, the conventional dose, is sufficient to prevent nerve impulses from stimulating muscle contractions, thus paralyzing the body.

Though curare affects muscles throughout the body, death geenrally results about five minutes after injection of hte drug, from paralysis of the chest and abdominal muscles used in normal breathing.

After injection, the body rids itself of about one-half of the drug within 24 hours, and the rest thereafter. Actually, the curare-induced paralysis usually lasts less than an hour and is temporary in the sense that the individual would recover normal muscle function if a mechanical respirator was operating during the paralytic period.

However, doctors who have volunteered to take curare and similar compounds under supervised experiments have described the ordeal as horrifying, knowing they were suffocating and near death. Despite these and other experiments, doctors had great difficulty in devising tests for identifying curare in the body. This situation enhanced the drug's reputation as a deadly poison that could not be detected even when a possible victim was exhumed.

Indeed, when Joseph C. Woodcock Jr., the Bergen County Prosecutor got a court order to exhume five bodies buried a decade ago, some forensic pathologists publicly said that the bodies would be too decomposed to make the venture worthwhile, and that the exercise would be a waste of taxpayer money.

Such were the imposing questions that confronted the medical detectives when they set about exhuming the bodies.

### Remains 'Well-Preserved'

On Jan. 13, grave diggers acting under court orders dug up the coffin containing the body of Nancy Savino, who was 4 years old at the time of her death in 1966.

Unlike many buried coffins, this one was air-tight and dry, which prevented rain water seepage that could have hastened the decomposition process. Dr. Baden himself was surprised. He said he saw "ar

amazingly well-preserved body appearing as if she had been buried only recently—her facial features were immediately recognizable."

Even the corsage that lay beside her body was well-preserved.

The remarkable state of preservation of the child's body was a key factor in Dr. Baden's attempt to reconstruct the cause of death, if possible. The child had died suddenly and inexplicably two days after an otherwise uneventful operation to remove her appendix and a section of small bowel that contained abnormal cysts.

The sutures still held the incision in place. Further, the bowel was still spliced by catgut sutures, which ordinarily dissolve in a living person after a few weeks by action of the digestive juices. The stump left in the bowel where the appendix had been removed was just as described in the surgical record.

Such anatomical detail enabled Dr. Baden to conclude that there was no apparent natural cause of death, which confirmed findings of the first autopsy, and to proceed toward his major task of detecting curare, if it were present.

For up to four hours—on the Savino child and in each of the other cases—Dr. Baden dissected and visually examined the organs for evidence of gross abnormality. Later he and his assistants spent dozens of hours peering at sections of each organ under the microscope for subtler clues to the cause of death.

Before Dr. Baden had opened the first exhumed coffin, he had asked cooperation from Dr. Dal Cortivo, who had trained at the Medical Examiner's office here and who is now chief toxicologist at the Suffolk County Medical Examiner's Office. Dr. Dal Cortivo said that he had originally expressed doubts about whether curare would remain stable in the buried tissues because they were subjected to unknown conditions over the span of a decade.

Dr. Dal Cortivo knew that curare could be detected in body fluids such as blood and urine. But these would have evaporated long ago. Little was known about the prospects of identifying curare in tissues, particularly decomposed organs. Though the odds were long and despite his skepticism, Dr. Dal Cortivo said he had agreed to cooperate. "It was a chance to learn something about an obscure drug," he explained. "It was a real scientific challenge."

Among the unanswered questions that Dr. Dal Cortivo faced were:

¶What happens biologically to tissue that remains in the ground for 10 years?

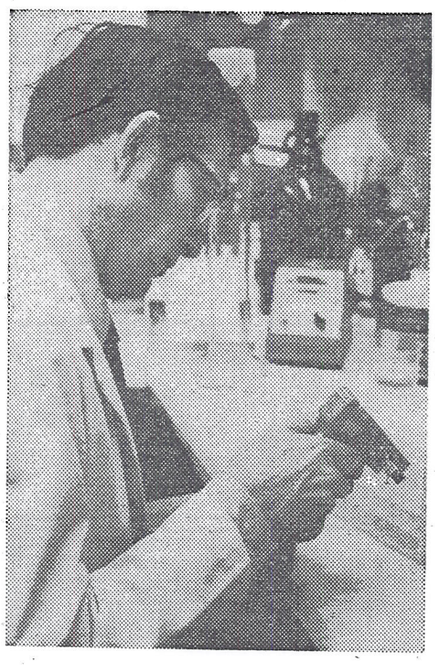
¶If curare was present at death, would it still be in the body now as curare? Or might it have disintegrated into other compounds? If so, which ones?

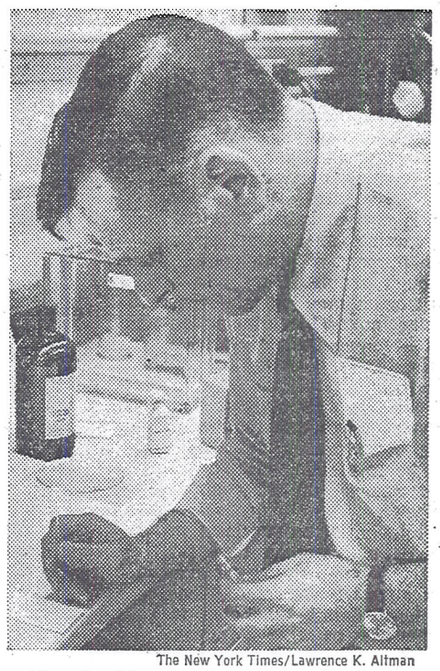
¶Even if curare was present, could so little be left that use of the most sophisticated modern technology would be incapable of detecting the drug?

¶What substances present in the earth or embalming fluid might chemically interfere with the tests and produce false results, either positive or negative?

Before Dr. Dal Cortivo drove to New York to pick up the Savino child's specimens, he had tested, as a control, samples of the earth and embalming fluid. He found no evidence of curare.

But in the tests, Dr. Dal Cortive found the embalming fluid to be acidic, which was fortunate because curare tends to be more stable in an acid solution. The body normally becomes basic when it decomposes. If curare were present, the fortuitous choice of an acidic embalming fluid may have helped preserve the drug.





The New York Times/Lawrence K. Altman

**Dr. Richard S. Matteo squeezes a laboratory handgun to deliver drops measuring one-thousandth of a cubic centimeter into test tubes as part of the test to detect curare in human tissue.**

**Also checking for the presence of curare, Dr. Leo A. Dal Cortivo places solvent on a plate to do a twin-layer chromatography test. He is chief toxicologist at the Suffolk County medical office.**

At Smithtown, the toxicologist set up a series of experiments to determine if curare could be detected in tissue. He added d-tubocurarine, the form of curare suspected of having been injected by Dr. X, into samples of tissue taken from other autopsied patients. He tested the first samples after an interval of a day or two. They were positive.

Would the results still be positive if the curare had been left in the tissue for two weeks? He tried that experiment. Again, a positive test result.

By the very chemical nature of d-tubocurarine, which gives it a positive charge, and by its molecular configuration, which makes it bulky, the drug tends to resist chemical extraction by many conventional solvents.

"It's a struggle to get curare out," Dr. Dal Cortivo said.

Throughout the preliminary experiments, Dr. Dal Cortivo modified a method developed in 1963 by Dr. Ellis Cohen, a Stanford anesthesiologist to chemically extract curare from tissue.

Dr. Dal Cortivo began extracting curare from the samples he picked up from Dr. Baden's autopsy. The toxicologist added water to slices of tissue about the size of a grape and weighing less than five grams, homogenized the mixture in a blender, then put the macerated tissue through a series of reactions with ether, methanol, dichloroethane and a stream of nitrogen gas.

First he tested the liver because as the organ that breaks down most drugs, it was most likely to reveal presence of the poison. Next he tested kindey tissue and then the lung.

At the same time, Dr Coombis, the New Jersey toxicologist, was going through similar procedures.

In two more chemical steps the toxicologist extracted entire classes of acidic and basic drugs that, if present, might have confused the test results.

### Other Technique Tried

Even then, Dr. Dal Cortivo did not know if the Savino child's tissue contained d-tubocurarine. To find out, he tried a technique called TLC — for thin layer chromatography—a variant of the paper chromatography test that students often do in high school chemistry courses.

Chromatography relies on physical and chemical characteristics of individual drugs in a solvent so that mixtures can be separated into their constituents as they flow along a strip of filter paper in paper chromatography or along a thin layer of silica gel in the TLC test.

In the TLC test, Dr. Dal Cortivo and his aides put three drops—a drop of the solvent, a drop of known d-tubocurarine and a drop of the unknown sample from the exhumed tissues—on the baseline of the silica gel plate. During the next hour, each substance leaves a streak as it moves up the plate by capillary action, the way water does when it climbs up tissue paper in a glass. Then the toxicologist sprays the plate with platinic iodide, which makes the known d-tubocurarine turn violet at a particular point.

If the spot from the unknown sample turns the same shade of violet at about the point on the plate, presumably that sample of exhumed tissue contains d-tubocurarine.

How could Dr. Dal Cortivo be sure that the spot represents d-tubocurarine if the test results are just tentative?

TLC, by separating out the components in a mixture, also acts as a purifier. The area around the violet spot presumably contains only curare; when subjected to further chemical extraction techniques, it should yield a purer concentration of d-tubocurarine.

Even so, a diagnosis of curare poisoning based simply on a positive TLC could be challenged by other scientists.

The clincher must come from a series of confirmatory tests, Dr. Dal Cortivo said, none of which was generally available when the Riverdell patients were buried.

### Additional Experts Test

Because Dr. Dal Cortivo had facilities to do just one of these three tests (gas-chromatography), he and Dr. Baden called on experts at Columbia and a commercial laboratory in California to do the other two —radio immuno assay and mass spectrometry. Dr. Coombis's team did the same tests independently in New Jersey.

The radio immuno assay (RIA) is an extremely sensitive process, capable of detecting billionths of a gram of a substance. (There are about 30 grams of water to the ounce.)

In the two dacades since the RIA test was developed to detect insulin at the Bronx Veterans Administration Hospital by the late Dr. Saul Berson and Dr. Rosalyn Yalow, it has been applied to an ever growing list of substances. In 1973, it was adapted for the detection of curare in fluids like blood and urine by Dr. Peter E. Horowitz and Dr. Sydney Spector at the Roche Institute of Molecular Biology in Nutley, N.J.

When Dr. Baden sought experts to do the RIA test on the exhumed bodies, he learned that only a small number of researchers had the biological materials needed to do the test and that none had used the method to detect curare in human or animal tissue. Also, he was unsure whether the technique would identify curare in tissue.

However, Dr. Richard S. Matteo, who was just about to try this step at Columbia as part of his research studies on the safety of using curare in children undergoing surgery, agreed to test samples from each patient for curare.

Less than a thimbleful of blood is needed to do the RIA test, which is based on an immunologic principle known as the antiger antigen-antibody re response. Antibody, the protein formed to ward off specific foreign material, reacts with antigen, the foreign invader. Curare antibody, formed by injecting small doses of curare into a rabbit, serves as a crucial component of the RIA test that is done in test tubes. There, curare binds with the rabbit antibody.

### Competitive Reaction

The test, in simplified terms, is based on a competitive reaction between "cold" (nonradioactive) curare—if any is present in a sample of exhumed tissue—and "hot" (radioactive) curare, and in turn between these curare antigens and the rabbit curare antibody.

From previous measurements, the researcher knows how much "hot" curare will react with the rabbit antibody in the test tube. When cold and hot curare are added to the same test tube a competitive reaction occurs. Both the cold and hot curare compete for the limited number of binding sites with the curare antibody.

As a result, any cold curare in the exhumed tissue would take up more binding sites, thus showing the presence of curare in the samples being tested.

The last crucial test now remaining to be performed, is the mass spectrometer, and while toxicologists used one in New Jersey, Dr. Dal Cortivo sent a set of tissues carefully wrapped in styrofoam by air freight to Finnigan Corporation in California.

The mass spectrometer is a device that, by electron bombardment, cracks or fragments a compound into particles that can be identified as they pass through a magnetic field. The machine, which costs up to $135,000, creates a set of chemical fingerprints because each compound cracks in its own way, and the toxicologist can match the fragment pattern of a sample of an unknown compound with that of a sample of the known drug that the computer recalls from its memory.

From the library of 40,000 compounds that toxicologists have compiled, "no two give the same pattern," Dr. Finnigan said. "If the mass spectrometer test is positive for a compound, the identification is absolute."