Over the past few years, the world has witnessed the final engagement in a long and costly war against smallpox. It now becomes increasingly clear that the war has been won, that smallpox has been totally and we hope irrevocably eliminated from among the plagues of mankind. The last stages of the war were necessarily global—that is, involving cooperation of many nations. The story is unusual in many regards, not the least of which is that it has a very happy ending. For this reason, it merits particular consideration by historians as well as physicians and biologists.

Smallpox is certainly an ancient disease. The earliest clinical material still extant is to be found on the mummy of Pharaoh Ramses V, who died about 1160 B.C. A study of the lesions on his face has led physicians to the conclusion that he must have died of acute smallpox. In ancient times the disease was epidemic in the Orient and in India. Curiously, its spread as an epidemic into Europe seems to have been delayed for reasons not entirely clear. It spread through Europe during the Middle Ages and soon assumed its place among the great epidemic diseases. Morbidity and mortality rates were appalling. It is estimated that the usual mortality in an epidemic ranged from 20 to 40% of affected individuals. It is generally believed that no more than 20% of the population of Europe escaped an attack of smallpox. Partially isolated islands were peculiarly vulnerable. It is claimed that one-sixth of the population of Ireland died within a single year of the disease, while 36% of the population of Iceland succumbed in 1707 in a similar epidemic. Although those who survived an attack of the disease were immune, a fact which was early recognized, they also were frequently hideously pockmarked—particularly trouble-

1 This paper was prepared at the invitation of Robert F. Acker, Executive Director, American Society for Microbiology. It represents an expansion of remarks made on 25 April 1977 in presenting Donald A. Henderson, Dean of the School of Hygiene and Public Health of The Johns Hopkins University, to the membership of the National Academy of Sciences on the occasion of the award of its Public Welfare Medal. Dr. Henderson has kindly agreed to append a postscript.
The success of these experiments led to the immunization of the grandchildren of George I. Some military leaders, including Fredrick the Great of Prussia, recognized the strategic advantages of having an army which was resistant to smallpox—a disease to be reckoned with in military campaigns.

Variolation was a procedure of significant hazard. Most of the published series of cases in the 18th century indicate that it carried a mortality of between 1 and 2%, although Robert and Daniel Sutton, father and son, reported a series of 2,514 consecutive variolations in England without a single death. The variolated individual, after all, was immediately at risk of contracting a generalized attack of smallpox. In addition, the variolated individual was a possible focus of a new epidemic of the disease. This was early recognized, and procedures were established to isolate the individual after variolation for appropriate periods of time. Undoubtedly, such attempts at isolation, however, were occasionally honored in the breach. Nonetheless, professional variolators appeared in all of the countries of Europe, and one must suppose that their successes outnumbered their failures.

The scene now shifts to North America, where in the city of Boston in 1721 an epidemic of smallpox erupted. The outstanding intellectual leader of the community was the Reverend Cotton Mather, remembered chiefly for his association with the witch trials in Salem. In the present historical context, Cotton Mather is revealed as a forward-looking and far-seeing leader. He apparently had access to the Philosophical Transactions of the Royal Society of London, probably through the good offices of Dr. William Douglass, a Scottish physician in the city of Boston, and here he read the papers treating of variolation. He called these to the attention of the approximately 30 physicians then practicing in Boston in the hope that they would muster an effective defense against the rages of the epidemic. Only one responded positively; this was Dr. Zabdiel Boylston, the son of a physician and himself a practicing surgeon. Defying the anger of his colleagues, including Dr. Douglass himself, and of the press, Zabdiel Boylston undertook a campaign of variolation, inoculating 280 citizens of Boston, of whom six died, possibly as a result of the inoculation. Of the nearly 12,000 persons residing in Boston at the time, about one-half contracted the disease during that epidemic and, of these, 844 succumbed. Thus, the mortality of the unvariolated population was 7%, while that among the population variolated by Dr. Boylston was only 2%. It is noteworthy that Boylston succeeded ultimately in convert-
ing the initially hostile medical community of Boston to his viewpoint, and even the recalcitrant Dr. Douglass finally admitted the virtues of variolation. Boylston’s success was appreciated in England, and in 1725 he traveled to London as a guest of its leading physician, Sir Hans Sloane, to report his experiences to the British medical practice. It is noted that during this stay in London he was accosted by a down-and-out young fellow American named Benjamin Franklin, who helped with the generous gift of 20 pounds—a gift which Franklin recalled in later life. Zabdiel Boylston returned to Boston and to the practice of medicine and surgery. He is recalled with affection by his grand nephew, John Adams, who, as a special privilege when a young lad, was taken occasionally to visit Uncle Zabdiel and to inspect his microscope and his telescope. Some interest attaches to the intensity of the public assault on Cotton Mather, proponent of variolation, and Zabdiel Boylston, its chief operator during the 1721 epidemic. There were vitriolic attacks by the press and personal attacks threatening the lives of these two gentlemen. An excerpt from Cotton Mather’s diary describes his evaluation of this assault.

July 16, 1721: At this time I enjoy an un-speakable Consolation. I have instructed one Physician in The New Method used by The Africans and Asiaticks, to prevent and abate the Dangers of the Small-Pox, and infallibly to save the Lives of those that it wisely managed upon them. The Destroyer, being enraged at the proposal of any Thing, that may rescue the Lives of our poor People from him, has taken a strange Possession of the People on this Occasion. They rave, rail, they blaspheme; they talk not only like Idots but also like Franticks, And not only the Physician who began the Experiment but I also am an Object of their Fury; their Obloquies and Invectives.

Cotton Mather was reacting to what was neither the first nor the last time when irrational public emotion attempted to dictate the course of scientific development. One wonders how the good Dr. Mather would have reacted to the “Obloquies and Invectives” hurled at the bold physician who dared to screen babies for the XYY karyotype in the same city of Boston 250 years later. Fortunately, in 1721, reason and science refused to succumb to public pressure. Mather’s performance may be held to vindicate his earlier, less distinguished record in regard to the epidemic of “witchcraft” in Salem. He was, in his time, one of the most prolific American men of letters and was honored by election as the first American member to the Royal Society of London.

The beauty of milkmaids is legendary. It was extolled by the Elizabethan poets, but few, if any, appreciated that this beauty was skin deep. It resulted from the fact that most milkmaids did not exhibit the pockmarking which was so very common among other women. The association between the absence of smallpox among milkmaids and the occurrence of a disease of cattle—cowpox or vaccinia—appears to have been missed for a long time. This pustular disease of cattle was transmitted to the hand of the milkmaid in the course of her work, where it resulted in a localized lesion and did not evoke a generalized eruption. What it did, however, was to confer effective immunity for a period of some years to the thus accidentally vaccinated milkmaid. The relationship between exposure to vaccinia and immunity to variola appears first to have been made by Benjamin Jesty, a British farmer, who deliberately transferred vaccinia to one or more humans in 1774. A similar procedure was carried out by schoolmaster Plett in Holstein in 1792. These experiments, however, had little or no general impact until the studies of Edward Jenner (1749-1823) a country practitioner of medicine who settled in Berkeley, in a rich dairy area. He had had excellent training; among his teachers was the great physician, John Hunter. It was with Hunter that Jenner shared his original ideas, conceived about 1780. His preceptor advised him, “Do not think, try.” Jenner disregarded the first portion of this recommendation and considered his problem for many years. Finally, in 1796, he performed his first vaccination on an 8-year-old farm boy, named James Phipps, with matter from a pustule on the hand of a milkmaid who had contracted cowpox. He subsequently challenged the boy’s immunity by variolation and found the immunization to be effective. Jenner was a minor poet and a humanist. He provided James Phipps, in later life, with a home around which he personally planted flowers.

The experiment is a brilliant example of targeted research. Jenner knew exactly what he wanted to accomplish, and after performing a single experiment he determined that he had hit a bull’s-eye. He continued to experiment along these lines and 2 years later in 1798 published his brochure entitled “An Inquiry into the Causes and Effects of the Variolae Vaccinae, a Disease Discovered in Some of the Western Counties of England, particularly Glouces tershire, and known by the name of THE COW POX.” It was an instant success. Within the 2 years that followed, vaccination was practiced in many countries of Europe, and by 1800 it was introduced in the United States.
were dipped into vaccinial pus, and these, upon drying, were shipped from place to place. It is certainly worth noting that active material survived the then slow passage across the Atlantic Ocean. Dr. Benjamin Waterhouse, the first professor of the theory and practice of physics at Harvard, received some vaccine from British friends and proceeded to vaccinate 7 of his own 13 children. Could this have been in the nature of a controlled clinical trial? He passed his information and some of his materials onto his good friend, Thomas Jefferson, who, with his usual enthusiasm for scientific advance, proceeded to vaccinate his entire household of family and slaves. Jefferson predicted that vaccination for smallpox "would finally extirpate that disease from the earth." At this time we are celebrating the fulfillment of this prediction.

Vaccination had obvious advantages over variolation. It produced a single local lesion, it produced significant disease in humans only in very rare instances, and it did not require quarantining of its subjects. Here, at last, was an almost ideal prophylaxis. Jenner was honored at home and abroad, and by 1804 a medal was struck in France commemorating the introduction of vaccination.

The history of the rapid acceptance of vaccination belies the claim that an excessive delay normally intervenes between the development of a new scientific device and its wide clinical adoption. When the device is truly useful, when it is quite obviously so, and when its early application reveals that it is relatively free of adverse reaction, then it appears to this writer that the application is often very rapid, the acceptance very general. When there is a significant delay between discovery and application, it frequently proves that the delay was justified.

By 1840, the advantages of vaccination over variolation had become sufficiently obvious to warrant the passage of a law in England making variolation a felony. Vaccination was practiced with increasing frequency, but progress toward the eradication of the disease was not smooth and major epidemics occurred from time to time. For example, during the France-Prussian War (1870) there were about 200,000 cases and 24,000 deaths from smallpox among the soldiers, while in the besieged city of Paris 18,000 people died of the disease.

In the early 20th century, massive vaccination programs in many countries led to the elimination of the disease, except for sporadic outbreaks, in the developed countries of Europe and America. In 1967, smallpox was still endemic in 33 countries, and by one estimate there may have been 10 to 15 million cases of the disease. In that year, the World Health Organization established a program directed toward the total eradication of smallpox. This was considered feasible for the following reasons: (1) the disease has no known animal reservoir; (2) there is no indication of a chronic carrier state for smallpox; (3) there is a very effective immunization procedure which provides a moderately enduring and high level of immunity; (4) the immunization procedure is both safe and easily carried out.

Headquarters were set up in Geneva, and Dr. Donald Henderson, then of the Center for Disease Control, Department of Health, Education, and Welfare, was detailed to the directorship of the program. Whereas his headquarters were in Geneva, the world was his laboratory for the big experiment which was about to be initiated. Clearly, success would be unlikely if countries which harbored the disease did not enter into the program. Such initially was the situation with Nigeria, which, under considerable pressure, finally agreed to participate. Teams of health officials, derived chiefly from the country under consideration but directed in most instances by physicians trained in the United States, France, Turkey, India, Brazil, Austria, and the U.S.S.R., were soon in the field vaccinating large numbers of the population.

The ultimate success of the program rested largely upon three innovations. The first of these was the replacement of the fluid suspension of vaccinia particles which had commonly been used by a freeze-dried preparation. This effected a very large increase in the stability of the product. The fluid suspension, for its continued preservation, had to be refrigerated, a condition which was difficult to meet in the field, particularly in certain underdeveloped countries. The freeze-dried preparation, however, was stable for many weeks at ambient temperatures. The second innovation related to the mode of delivery of the vaccine to the susceptible subject. The old scratch techniques were at first replaced by the use of a jet gun which sprayed the particles into the skin at very high velocities. This device permitted the very rapid vaccination of large numbers of people. However, although ingenious and highly efficient when it was in operating condition, the jet gun turned out to require frequent servicing. Such servicing was often difficult to provide. For this reason, another device, both inexpensive and simple, was needed, and this was provided in the form of the two-pronged needle. Vaccinators, it was found, could easily be trained to use this simple instrument which was cheap, sterilizable, and disposable. The third
innovation was in many ways the most interesting. The initial concept of the campaign was to vaccinate a very substantial fraction of the world population. Although this would certainly have been effective, it would also have been expensive and time-consuming. It was discovered that essentially the same effect could be achieved by surveillance of the population, prompt identification of new cases, and the containment of the disease in the vicinity of new cases by construction about each new case of a barricade of immunized individuals. This novel, epidemiological technique became known as surveillance containment. It took advantage of the fact that the rate of spread of smallpox in a typical population was slower than had been anticipated and that the rate at which a skilled team of vaccinators could spread immunity was more rapid than the rate of spread of the disease. In many countries the application of this technique permitted the eradication of smallpox with the vaccination of only a small fraction, as low as 6%, of the total population. To ensure that the surveillance was effective, a system of bounties was established. As smallpox became progressively less and less frequent, the bounty to be paid the reporter of a new case increased. The most recent quotation for the reporting of a new case of smallpox is $1,000.

Whereas Americans seem to have dominated the program, it was in fact international. The Soviet Union provided a very large fraction of the freeze-dried vaccine which was used, and physicians and health officers in each country participated actively and in large numbers. The total cost of the program over a 10-year period, however, was surprisingly modest and is estimated to have been less than $100 million. About 25% of this funding came from United States sources.

By 1976 it was clear that the outcome would be successful. In that year it was announced that smallpox had been eliminated from the world with the exception of the Horn of Africa. In October of 1975 the last Asiatic victim of smallpox was seen in Bangladesh. This was also the last case of variola major, the more lethal form of the disease. Thus, 10 years after initiation of the World Health Organization campaign, smallpox persevered only in its minor modification and solely in one area of the world. Ethiopia and Somalia comprise a difficult and inaccessible terrain at best. At this time the problem was complicated by bitter border warfare. Nonetheless, the vaccinators continued in their invasion, and in October 1977 they discovered and successfully treated the Somalia native, Ali Maow Maalin, who will probably prove to be the last victim of smallpox on the surface of this globe. (Some 11 weeks after the completion of this manuscript, I learned of a case of variola major in Birmingham, England. The disease, first recognized on 25 August 1978, was confined to a single case involving a medical photographer in an institute in which variola virus was being cultivated. A similar outbreak of laboratory infection had occurred in this same institute in 1966. The present victim was last vaccinated at that time. Inadequate immunization and an apparent breach in microbiological technique both contributed to the present unfortunate episode. The surveillance containment procedure with vaccination of all known and suspected contacts of the individual appears to have contained the disease. This episode will certainly lend support to the view that all or most laboratory stocks of variola virus scattered over the countries of the world should be destroyed.) Since that time, surveillance has of course continued, and despite the increasing bounties offered for the detection and reporting of new cases, none has been forthcoming. The procedure calls for a 2-year active and fruitless search since detection of the last case, after which there will be a final review by a team of experts before the pronouncement of ultimate victory.

There is a high level of confidence among
the experts in the field that no new cases of variola are likely to appear at this time. This is based upon experience garnered from many countries where smallpox has been eradicated. In no instance, after a 6-month lapse, has a new case appeared except by overt importation. Undoubtedly, we shall wait until 1979 before an official announcement of final victory is published.

In the past few years we have witnessed the transition of variola from the position of an endangered species to that of an extinct species. The disease is gone. We are told that there are scattered over the world a few deposits of variola virus, but these reside in the deep-freeze chests of investigators rather than in the tissues of human subjects. The ultimate disposal of these laboratory materials is still under consideration. They pose no serious hazard, however, since adequate stores of freeze-dried vaccinia vaccine are also being preserved.

Postscript

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26 October 1977 marks the date of onset of the world’s last known case of smallpox—just 10 years, 9 months, and 26 days after the World Health Organization’s intensified global eradication program began. More than 20 countries have already stopped their programs of smallpox vaccination. By 1980, the remaining phases of the program to confirm eradication will have been completed, and then smallpox vaccination throughout the world will cease. Apart from preventing untold suffering and blindness, the achievement of smallpox eradication will result in annual savings to countries throughout the world of more than $1,000 million.

More important than the achievement of eradication is the impetus and confidence which the program has given to countries throughout the world to undertake more ambitious programs to prevent disease. Rapidly gaining momentum is a World Health Organization global program to vaccinate children against the major childhood illnesses for which good protective vaccines are available—diphtheria, whooping cough, tetanus, measles, and poliomyelitis.

Smallpox has been a remarkable episode in the history of our civilization. It has contributed such words as inoculation and vaccination to our language and such ideas as immunity and virus to our thinking. It is, to date, the first and the only disease of humans which, by the conscious and deliberate effort of humans, has been eliminated from the face of the earth. This would appear to represent the highest goal of medical science. It sets, therefore, a model of achievement which may become an appropriate target in other disease situations. Measles and poliomyelitis suggest themselves as possible candidates for global eradication. In another sense, the several vitamin deficiency diseases are also susceptible of elimination, although in this situation surveillance will have to continue indefinitely. Up to the present time, no chapter in the history of medicine has come to a more satisfactory conclusion than has that one entitled smallpox.
The Penicillin Saga Remembered

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In recounting one of the great human dramas of our time, the best I can hope for is to outline the main threads of development as seen and experienced by one individual working in a team primarily at the Northern Regional Research Laboratory (NRRL) in Peoria, Ill., but having close contacts in Wisconsin and other universities and in many pharmaceutical laboratories here and abroad. I am not a chemist, engineer, or physician, so I shall of necessity have to omit, or treat in minimal fashion, many important chapters of the story I propose to tell. Also, if I tend to mention some names repeatedly, as I will, and fail to include others, as I must, keep in mind that there would be no penicillin saga to relate were it not for the close and unselfish collaboration among hundreds of individuals in the United States and Great Britain during a very critical period in our national histories.

And now with that brief preamble, may I call your attention to Fig. 1, for it expresses in poignant terms just what the penicillin saga is all about—a young 4-year-old boy with a severe Staphylococcus infection photographed at zero time and again 11 days later after treatment with a very impure and still untried emergent drug, penicillin (ex Abraham et al., Lancet 241:177, 16 August 1941).

Fig. 1. Young boy with severe Staphylococcus infection at beginning of treatment and on day 11 of treatment. (ex Abraham et al., Lancet 241:177, 16 August 1941).

(1929), modestly announcing that an antibacterial substance from cultures of a Penicillium was useful in isolating Bacillus influenzae. That was the beginning, and we should in no way minimize its importance, for Fleming did discover and apply the name penicillin to the active principle in his culture broths; he did find that it was essentially nontoxic to laboratory animals; he did show that it was especially active against gram positive bacteria; and he did speculate that it might have utility in combating certain medically important pathogens such as Staphylococcus and Pneumococcus. That is about as far as he went—how could he possibly envision what was to follow?

But let’s digress for a moment and consider the man (Fig. 3) whose name was to become a household word a decade later. He was at Boulogne during World War I. He was appalled by the slow convalescence of the wounded soldiers that he had to treat and distressed at the limited efficacy of the antisep- tics then in use. He was ever a believer in the importance of one’s body defenses, and especially so after he discovered a bacteriolytic substance, lysozyme, in human tears, saliva, and sputum in 1922. Contamination of...

Fig. 2. Culture plate showing the dissolution of staphylococcal colonies in the neighborhood of a Penicillium colony. (ex Fleming, Br. J. Exp. Pathol. 10:226, 1929).

Fig. 3. Alexander Fleming at NRRL, July 1945 (NRRL photo).
his Staphylococcus plate by a mold was an accident; but Fleming’s recognition of a potentially important phenomenon was no accident, for Pasteur’s observation that “chance favors the prepared mind” was never more apt than with Fleming and penicillin.

Three years later Harold Raistrick, an outstanding organic chemist and coauthor of more than 100 papers on the biochemistry of molds, took up the study and with Clutterbuck and others published a paper on, among other things, “Fleming’s Penicillin.” In the meantime they had the mold correctly identified by Charles Thorn, who diagnosed it as Penicillium notatum, not Penicillium rubrum as cited by Fleming; they reported that penicillin was unstable in acid and alkali and that it was present in very small amounts; and they even attempted to interest some physicians in testing their “concentrate” on some patients but to no avail. They also isolated a yellow pigment, chrysogemin, about which I shall comment later.

Some additional information was published by Roger Reid, then at Pennsylvania State College, in 1933 and 1935. But that is about all, and Fleming’s penicillin was virtually forgotten. In fact, when Thorn, at the International Microbiological Congress in New York in 1939, asked Fleming what had become of his penicillin, he was told, “I forgot about that some years ago.”

With the outbreak of World War II imminent, the need for drugs effective against battle wounds (staphylococci, clostridia, etc.) was recognized, and Howard Florey (Fig. 4) and a team at Oxford University, who incidentally had been studying lysozyme, dusted off Fleming’s penicillin. Associated with Florey were such men as E. P. Abraham, Boris Chain, Norman Heatley, and others, later joined by Florey’s wife, Mary, who was also a physician. This was the real beginning of the antibiotic era, and very important work was reported, particularly in 1941. Methods for growing the mold had been developed by Abraham, Gardner, and others, modeled after those of Raistrick, and procedures for extracting and concentrating penicillin had been improved. Chain had accumulated appreciable information regarding the chemical properties of penicillin; a somewhat mystical but very essential assay had been developed by Heatley; and the Floreys had conducted animal experiments, the first of which is shown in Fig. 5, and had actually treated some patients with promising results, including the boy previously shown. However, there were serious disappointments; for example, while successfully treating a London bobby, their supply of penicillin ran out and the policeman died of staphylococcal pyemia. There were untold production difficulties as well, and concerning these Florey et al. tell an interesting story in their two-volume work, entitled Antibiotics (1949). The mold was cultivated as surface cultures, the only method then known, and the number of Erlenmeyer flasks required to produce the reasonable amount they needed for further purification was prohibitive. They overcame this in the following manner, as I quote:

It may be amusing to recount the evolution of the first vessels. Apart from the usual laboratory ware, trials were made with various kinds of glass and enamel domestic dishes and utensils, and biscuit and other tins (both with and without a coating of lacquer or varnish), but it was found that the old-style bedpan with a side-arm and lid was an ideal culture vessel, providing a relatively large surface area over a shallow layer of fluid and with a side-arm for inoculation and withdrawal. Unfortunately when an effort was made to procure 600 of these vessels it was found that such a large number could not be provided as they had been replaced by a more modern streamlined structure without a lid. At the time they were required the Battle of Britain had been won but the country was being subjected to heavy bombing so that it was difficult to secure supplies of any sort. Glass vessels could not be made within a reasonable time, but Messrs. J. Macintyre & Co., of the Staffordshire pottery industry, undertook to make special rectangular porcelain vessels, fitted with a side-arm,
which could be readily stacked in incubator and sterilizer. To overcome transport difficulties Heatley borrowed a van and drove 200 miles to fetch the first consignment. He returned with them in a snow-storm on 23 December and they were first sown with *Penicillium notatum* on Christmas Day, 1940.

The bombing of Britain made it impossible to envision substantial production there; with support from the Rockefeller Foundation, Florey and Heatley came to the United States in July 1941 to seek help for the manufacture of penicillin. They stopped in New York and visited some of the pharmaceutical firms in the environs; although one cannot say they were given a brush-off, they were not exactly received with open arms. The reasons are quite clear. As a drug, penicillin was at a very early experimental stage, and yields at that time rarely exceeded 4 units per ml of culture broth. In any case, the Foundation sent them to the National Academy of Sciences in Washington; President Jewett sent them to see Charles Thorn, a member of the Academy and the world’s authority on *Penicillium* (Fig. 6); and Thorn, in turn, recommended that they go to the Fermentation Division of the newly created NRRL in Peoria, Ill. Official arrangements were made through the acting chief of the Bureau of Agricultural and Industrial Chemistry, Percy A. Wells, and the Englishmen arrived in Peoria on a Sunday afternoon and came to the laboratory the following day, 14 July 1941. Work was initiated immediately.

Why Peoria? you may ask. There were good reasons.

1. Thorn was being moved from Washington to Beltsville and was about to retire.
2. The U.S. Department of Agriculture’s Fermentation Laboratory, formerly at Arlington Farm (where the Pentagon now stands), had been dismantled and moved to Peoria the previous year, and on its staff were men experienced in mold fermentations: Andrew J. Moyer, George E. Ward, Lewis B. Lockwood, H. T. Herrick, O. E. May, and others, together with Robert D. Coghill, newly appointed Head of the Fermentation Division.
3. Thorn’s very substantial collection of molds had gone with me to Peoria, also, and this was to be the potential source for many of the microorganisms that we would study.

At NRRL, research on penicillin was initiated at once, and it was mutually agreed among the English and ourselves that this would focus on the following three primary objectives:

1. To formulate, if possible, a more productive substrate-improvements, had been possible in earlier fermentations, e.g., citric acid.
2. To develop, if possible, a submerged method for producing penicillin—this had been done for gluconic acid, using *Penicillium chrysogenum* and *Aspergillus niger*.
3. To find or develop a more productive culture—in previous mold fermentations, strains better than the original had always been found. Still, the Fleming strain of *P. notatum* was then the only mold known to produce penicillin, so our work began with it and in surface culture.

Florey remained in Peoria a few days and then returned to England via New York. Heatley (Fig. 7) stayed for several months to acquaint us with production methods as practiced in England and, more importantly, to teach us how to assay for the presence and titer of penicillin—without which we would have been hopelessly lost. Looking back, one may tend to mock surface culture methods, but keep in mind that this was then the one method known for producing some penicillin.

In time, singular success was realized in attaining each of the three aforementioned goals. Before considering these, perhaps we should speak briefly of the assay based on the so-called Oxford unit (OU) that depended upon the diffusion of penicillin through an agar plate preseeded with *Staphylococcus aureus* (Fig. 8) and was defined as follows: “that amount of penicillin which when dissolved in 1 ml of water gives the same inhibition as this (arbi-
Fig. 8. Early penicillin assay plate showing cylinder method (NRRL photo).

If this sounds like the blind leading the blind, it is not far from the truth, for new standards had to be set up quite frequently, and each had to be based on a prior one.

It was not until October 1944, more than 3 years later, that an international unit based upon weight was established. The standard was based upon pure sodium penicillin G; by definition, 1 mg was to equal 1,667 international units, or 1 unit = 0.6 μg, which by design was set to approximate the OU, about which there was then a vast literature. The pooled sample upon which the international unit was based had been recrystallized by Frank Stodola and J. L. Wachtel of NRRL and assayed 1,650 OU/mg, which was not bad agreement. Figure 9 provides a dramatic comparison between equal amounts of pure penicillin and the crude drug (40 to 50 OU/mg) used in early therapeutic studies.

Pure penicillins other than sodium penicillin G have, of course, different units per milligram, but all were interpreted in terms of that standard. With modifications, e.g., substituting filter disks for cups, the zone of inhibition assay is still used by the Food and Drug Administration.

Now to return to the work at NRRL and elsewhere in the United States. Because of his past experience with mold fermentations and particularly the production of gluconic acid, Dr. Moyer was assigned primary responsibility for improving the culture medium; progress was dramatic. By Christmas of 1941, yields were up to 40 OU/ml, a 10-fold increase; the pharmaceutical industry was definitely interested! Additionally, the United States was then at war. Some work had been done meanwhile at Merck, at Pfizer, and particularly at the Squibb Laboratories, for Geoffrey Rake was able to return to us in January 1942 a version of the Fleming culture, accessioned as NRRL 1249, which was superior to the one then in our possession. This became the basis for future surface culture studies.

Variations of culture media were studied, and the introduction of corn steeping liquor (previously used in the gluconic acid fermentation) proved to be the single most important ingredient, particularly when combined with lactose (which was slowly utilized) and some inorganic salts. This culture medium, with various modifications, remained for many years the one most used in industry, first for surface production and subsequently at lower nutrient concentrations for submerged production as well.

By careful selection of monoconidial cultures from NRRL 1249, a substrain, 1249.B21 (see Fig. 12), was obtained within a few months, which yielded up to 180 to 200 OU/ml in flasks in laboratory experiments and up to 100 OU/ml or more in bottles used in the factories (Fig. 10). It was the strain generally used for this purpose, and inefficient as the culture and method may seem in retrospect,
this strain and the bottle method were responsible for the production of the penicillin upon which the efficacy of the drug was firmly established by the Floreys in England and by Herrell, Keefer, Finland, Mahoney, and others in this country. The type of penicillin was penicillin F, to which I will return later. Figure 11 is a comparison of the curative rates in World War II with those in World War I for two types of battle casualties, the improvement being due in major part to penicillin.

In passing, I should say that a modification of the surface method was seriously investigated by researchers at Parke, Davis & co., prompted no doubt by their prior experience in making a digestive aid, Takadiastase, by growing Aspergillus oryzae on bran. Penicillium grew well, and the mold produced some penicillin, but the elution was difficult, and they soon joined others of that day in turning to bottle production. Another and more successful variation was actually practiced for a time at an experiment station in Hawaii. Gauze floating on nutrient broth was inoculated with P. notatum; when it had grown, the gauze was lifted off and applied directly to superficial wounds.

Word of this might have gotten around, or a lot of people came up with the same idea at about the same time. In fact, "homemade penicillin," a favorite topic for the Sunday supplements, so threatened to become a reality that Dr. Coghill and I felt constrained to place a note in the Journal of the American Medical Association (24 December 1943) warning that there were scores of blue-green penicillia of which very few were known to produce penicillin, and what some of the others might produce no one really knew. Little did we know how prophetic we were, for even the term "mycotoxin" was still in the shadows. Penicillin was not just news in 1943 and 1944—it was big news; the press seemingly reported every civilian request for the drug—occasionally available, usually not, but good copy nonetheless.

Let us return to the main thread of our story.

Fig. 12. Fleming's P. notatum NRRL 824 (left) and a derivative strain, NRRL 1249.B21, used for the production of penicillin in surface cultures (right) (NRRL photos).

For reasons still unknown, neither strain 1249.B21 nor any other derivative of the Fleming culture (Fig. 12) was ever found to produce interesting yields of penicillin in shaken flask or other types of submerged culture. Still, all realized that if the drug was to be produced in the amounts then being demanded, some type of tank process was imperative. Knowing that molds differ in their potential, it was reasoned that by careful search and screening we might find a culture of P. notatum that could be adapted to production in submerged growth. Such a strain, NRRL 832 (Fig. 13), originally from Biourge's laboratory in Belgium, was found among the cultures then maintained in our collection. When grown submerged, it produced much lower yields per milliliter (ca. 40 to 50 OU) than did 1249.B21 when the latter was grown in surface culture, but even this amount was deemed advantageous; it produced primarily penicillin G, a more desirable drug than penicillin F. As rapidly as feasible and as fast as they could obtain the
scarce but necessary stainless steel for fermentation tanks and ancillary equipment, most of the manufacturers began to phase out their bottle plants and replace those with factories designed for submerged production. The reason was as simple as this: one 10,000-gallon tank was the equivalent of 60,000 to 70,000 2-quart milk bottles, with tremendous savings in labor and in losses from contaminated cultures— if they operated pure culture tank fermentations, which, by the way, was no easy task. *Penicillium* is aerobic, and huge quantities of sterile air had to be provided while the whole content of the tank was continually and vigorously stirred. Too great of credit cannot be accorded the engineers and microbiologists who together achieved these goals. Yields of up to 80 to 100 units per ml were in time obtained, and for a year or more NRRL 832 was the culture used for the bulk of an ever-expanding production. Attempts to produce more productive strains by mutation of NRRL 832 proved generally unsuccessful in our laboratory and elsewhere. Translating the results from shaken flasks to small and then to larger and larger tanks presented many difficulties in providing adequate aeration while maintaining asepsis. Furthermore, what was optimal for small vessels generally required modifications as tank volumes increased and their relative proportions changed. Nonetheless, through close cooperation among many research laboratories—government, university, and industry—these problems were resolved. Although I suspect that you are all familiar with the multistory fermentors now used in the antibiotic industry, I think we might look back four decades and see with George Ward the modest beginnings from which they sprang (Fig. 14).

The search for new and improved cultures was perhaps the most dramatic and fruitful of the three approaches, albeit its success was firmly grounded in the prior development of the corn steep liquor-lactose substrate and the demonstration that penicillin could be produced in aerated and stirred tanks. With NRRL 832 having been found among the cultures then in hand, a very determined search was begun to isolate even more productive cultures from nature. To this end we asked one and all to send in samples of soil, moldy grain, fruits, and vegetables; for some months in 1942 through 1943, we employed a young lady to scour the markets, bakeries, cheese stores, etc., of Peoria for samples bearing blue-green molds—a lady who did her job so well that she became known as "Moldy Mary." The irony of it is that the cantaloupe that yielded the bonanza strain NRRL 1951 came not from a fruit market in her search but from a Peoria housewife who brought the melon to the laboratory. The story caught on, the press loved it, and it became one of the favorite *antecedotes* in the folklore of penicillin. Perhaps it is just as well, for the cantaloupe was not a local melon, and it must have come from a market 2 or 3 days earlier. There were other interesting incidents, as well. An Arizona rancher sent in a beautiful green lichen-covered rock, thinking it was *Penicillium*, and said that he could send tons like it to speed along the project about which he had read. We also enlisted the help of the armed services; I remember well some of the packages sent in via the Navy and Air Force from faraway places and often wondered what the reactions of the noncommissioned officers (or privates) might have been when they were sent out to gather dirt for some characters in Peoria! All molds suspected of being either *P. notatum* or its close relative, *P. chrysogenum*, were isolated and put through a very simple preliminary screening procedure, with NRRL 832 and NRRL 1249.B21 grown as parallel controls (Fig. 15).

**Fig. 14. Early fermentors for mold fermentations used by George Ward (shown), and others: rotary drum fermentors (left) and small, upright aerated tanks that replaced them (NRRL photo).**

**Fig. 15. Simple screening test. Numbered plugs were cut in radial lines from edge of mold colonies and placed on plates preseeded with *Staphylococcus aureus* (NRRL photo).**
When first isolated, the cantaloupe strain of *P. chrysogenum*, NRRL 1951 (Fig. 16), gave submerged yields approximating those of NRRL 832 and was pigmented intensely yellow, an appearance that was then generally correlated with good penicillin yields. However, its superiority was not revealed until subcultures were isolated from a plate upon which mycelial growth from a week-old shaken culture had been streaked. It was singularly variable, and subcultures were picked from areas of differing growth patterns and numbered A, B, C, D, etc. Of these strains, B was the best; from this, a series of monoclonal cultures were isolated and tested for production in surface and submerged cultures. In both tests, one isolate, 1951.B25 (Fig. 16), seemed to be slightly superior; this became the basis of intensive study in our laboratory and elsewhere, for yields of up to 250 OU/ml could be obtained in small tanks. It was sent to collaborating laboratories and to manufacturing companies in April and May 1944. Demands for penicillin at this time were unbelievable, and the Office of Production Research and Development of the War Production Board set up projects at the University of Wisconsin, Stanford University, the University of Minnesota, and the Carnegie Institution in Cold Spring Harbor as the word went out that a million dollars would be a cheap price for a still better culture. It was not long in coming.

Milislav Demerec at the Carnegie Institution irradiated conidia of NRRL 1951.B25 with X rays, isolated survivors, and sent them to the University of Minnesota, where they were surveyed by Stacy French, John Ehrlich, and others. A dozen or so of these were selected as probably superior and forwarded to the University of Wisconsin, where they were first examined by Elizabeth McCoy and associates in the Department of Bacteriology and then sent to W. H. Peterson and Marvin Johnson in the Department of Biochemistry for evaluation in small tanks. One of this small group of cultures seemed to be clearly superior, strain X-1612 (Fig. 16), for this gave yields of up to 450 to 500 OU/ml, which were subsequently confirmed in a 60-gallon fermentor in Peoria. In turn, strain X-1612 was subjected to UV irradiation by Myron P. Backus and J. F. Stauffer in the Botany Department, and they came up with a still better culture, Wis. Q-176 (Fig. 16), capable of producing up to 900 OU/ml with basic cultural requirements essentially unchanged from the original Moyer medium. Jubilation knew no bounds—but not for long. It soon developed that, although the unitage was high, Wis. Q-176 was producing not penicillin G but a different and less stable antibiotic, penicillin K, which in vivo failed to retain satisfactory blood levels.

Fortunately, this adverse situation was soon corrected, for at that time phenylacetic acid was known to be part of the penicillin G molecule; Dr. Coghill (Fig. 17) and others reasoned that the production of penicillin might be redirected by adding this compound to the culture broth. This was done, the high yields were retained, and the penicillin was primarily the desired penicillin G. The addition of precursors to fermentation substrates became accepted practice and has remained an area of intensive research since that time.

The work at Wisconsin continued for several years with steady improvement in yields by careful selection of natural variants and the production of mutants by exposure of conidia from progressively better strains to UV light or nitrogen mustard compounds; as these better cultures were obtained by the mycologists and proven by the biochemists, they were released to industry. Also important was the isolation by Backus and Stauffer early in their series of a so-called pigmentless strain that was a good penicillin producer but lacked the capacity to produce "chrysogenin," Raistrick’s yellow pigment that had been a welcome indicator of good penicillin yields but a bane for the people

![Fig. 16. *P. chrysogenum* NRRL 1951 and cultures derived from it: 1951, wild type; 1951.B25, a "second generation" selection; X-1612, X-ray-induced mutation of the preceding, by Demerec; Q-176, UV-induced mutation of X-1612, by Backus and Stauffer (NRRL photos).](image-url)
who had to separate it from the pigment-free drug in the recovery process.

Needless to say, individual industries took these Wisconsin cultures and pursued their own programs for further strain improvement. More recently special laboratories have emerged to mass screen for even more productive strains or, as in the case of Panlabs, to supply manufacturers with known superproductive cultures—cultures that I am told produce up to 50,000 units per ml (≈ 30 mg of sodium penicillin G per ml), a far cry from the 4 units per ml with which we began! While subjected to untold mutations and selections during the past 35 years, it can be said, insofar as I have been able to determine, that the cultures now being used for penicillin production throughout the world stem from the cantaloupe strain isolated in Peoria in July 1943.

Many important developments have occurred during the ensuing years, but I do not have time to consider these in the manner they merit. Rather, I must be content to mention a few, and these all too briefly.

Along the way an important discovery was that by adding the precursor phenoxyacetic acid to the culture medium a new and valuable penicillin, phenoxymethyl penicillin, or penicillin V, could be produced. This was particularly advantageous for the patients since it could withstand gastric juices and hence could be taken orally. The discovery of the drug is credited to Otto K. Behrens of the Lilly Laboratories, while its acid stability was discovered by two Austrian scientists, Brandl and Margreiter. Since that time, penicillin V and penicillin G have been the two major natural penicillins manufactured and prescribed.

During the past 20 years, the so-called semi-synthetic penicillins have come into very wide use. For a while the penicillin nucleus per se was produced by fermentation, and selected side chains were added by chemical processing to make drugs with specific properties. More recently, the normal side chain of penicillin G (or penicillin V) has been removed by either chemical or enzymatic hydrolysis and another has been added. Some 30,000 of these semi-synthetic penicillins have been produced; of this number, a very small percentage have come into therapeutic use. Ampicillin and amoxicillin may be cited as representative of these newer penicillins that are especially useful because of their activity against gram-negative bacteria and, more importantly, against strains of gram-positive species that have become resistant to the older drugs.

Another very important development of the past 20 years has been the slow emergence and then a rapid expansion in the manufacture and use of the cephalosporins. These drugs are much like the penicillins in their basic structure; substitutions can be made to construct drugs which are a very useful alternative to the penicillins or for use when the penicillins are no longer efficacious. Most of these are obtained from the mold Cephalosporium acremonium by hydrolyzing a natural cephalosporin, cephalosporin C, and then by chemical synthesis constructing the drugs used clinically; some may be made by stripping the side chains from nuclei of penicillin G or penicillin V and adding more desirable ones to produce drugs such as cepalexin.

During this talk, I have said nothing about the streptomyces and the many useful drugs that have been and still are derived from them: streptomycin, the tetracyclines, erythromycin, gentamicin, kanamycin, and lincomycin, to name some principal ones. This is not because of their lesser importance. It is because they were secondary to penicillin in their genesis; it is because in their development and manufacture the same general procedures and equipment were used; and finally it is because after 38 years penicillin in its many current formations still represents the most widely used and the least toxic of all the antibiotics yet discovered.

For several years I plotted the monthly production of penicillin in the United States, which
by 1951 had reached about 25 to 30 trillion units. I regret that I have not kept the graph up-to-date, but I can say that according to the U.S. International Trade Commission the production of penicillin, exclusive of the semi-synthetics, was 384.5 trillion units for April 1978, and for the year 1977 the grand total was 3.875 quadrillion units, again not including the semisynthetics (even at 0.6 µg/unit this represents a very considerable amount). Amazing as the increase in penicillin yields over the past 35 years has been, the decrease in price has been even more dramatic; whereas 100,000 units sold for $20.00 in 1943 (when it could be obtained), the current market price is about $23.00 per kg (= 1.6 × 10^6 units).

To achieve this end, let it be said that the penicillin saga was made possible by teamwork: teamwork within the several institutional groups and among different groups, whether in government, universities, or industry. For example, the Oxford group included microbiologists, biochemists, chemists, and physicians; the Peoria group was similarly diversified (except for physicians); and both enjoyed the full cooperation of other participating groups; while during the war the whole endeavor was coordinated through the Office of Scientific Research and Development with the full support of the War Production Board.

The pioneering work on penicillin did not go unrecognized: Fleming, Florey, and Chain were awarded Nobel prizes in 1944, the same year in which the two Englishmen had been knighted by King George VI. Additionally, the Oxford team was cited by the Lasker Foundation in 1953, and this citation stands today engraved in pink granite in a small rose garden at Oxford University (Fig. 18). The Peoria team was given the Lasker Award in November 1946 (Fig. 19), followed by the U.S. Department of Agriculture’s Distinguished Service Award a year later.