Code Name Bacinol

Dutch microbiologists, working in secret during the last months of WWII, developed procedures for making penicillin

Marlene Burns, J. W. Bennett, and Piet W. M. van Dijck

Among microbiologists, The Delft School is well known for its contributions to the development of general microbiology through the legacy of Martinus Beijerinck, Albert Jan Kluyver, and Cornelius Van Niel. Many scientists from the Delft tradition became industrial microbiologists, and some of them were employed by the Nederlandsche Gist-en Spiritusfabriek, the Netherlands Yeast and Spirit Factory (NG&SF), one of the oldest fermentation companies in the world.

When Germans occupied the Netherlands during World War II, a team at NG&SF secretly isolated, characterized, and produced penicillin—working under the code name "Bacinol." Although "penicillin" now refers to a familiar group of β-lactam antibiotics, it initially was used to describe "mold juice" with antibacterial properties—in essence, a crude filtrate that contained one or more β-lactam metabolites and their impurities.

This 18-month project, which was completely independent of the acclaimed American and British efforts to produce penicillin, was conducted under far more challenging circumstances, without access to the scientific literature, corroborating data, and much in the way of valuable fermentation equipment. Moreover, amid the roundups and deportations taking place throughout western Europe, a Jewish physician, who was interned in a transit camp, provided key information to help the NG&SF team effort. Although this story is little known, parts of this narrative are more like components of a thriller than an undertaking in industrial microbiology. We base this article on interviews with surviving participants, archival materials, and other published records.

The Dutch Faced Many Difficulties during WWII

Soon after German troops invaded the Netherlands on 10 May 1940, the Queen, the Prime Minister, and other ministers fled to London, and the Dutch army surrendered. Instead of a military government, Hitler appointed an Austrian Nazi, Arthur Seyss-Inquart, as the civilian Reichscommissar to collect money and materials and to oversee labor to supply the German war machine. During the early months of occupation, the Dutch economy briefly improved and Dutch management largely stayed in place.

Within less than a year, however, the Nazi occupation imposed increasingly stringent conditions. In November 1940, all Jewish civil servants, including tenured university professors, were dismissed. Soon after, many Jewish properties were confiscated, and increasing numbers of Jewish citizens were arrested and sent to Westerbork, a transit camp near the German border that served as a conduit to eastern concentration camps. Thereafter, the Dutch economy was intensively exploited. Over 100,000 men were taken to Germany for labor service, and some 25,000 Jews went into hiding.

As the tide turned against the German armies in 1943, following defeats in North Africa and Stalingrad, Dutch living conditions became increasingly harsh, with strict rationing of food and fuel. After the Normandy invasion in June of 1944, it was expected that the liberation of the Netherlands would soon follow, but the majority of the Netherlands remained under Nazi control. In retaliation for a Dutch railroad strike, German authorities ceased almost all shipments of coal and food to civilians. Electrical power became erratic, and rations were reduced to as few as 500 calories per day. To make

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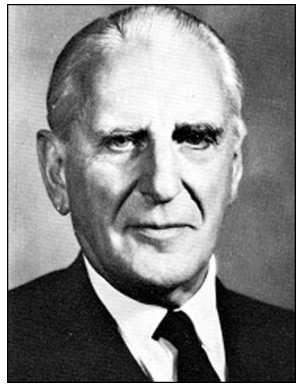
Chronology of selected important events in penicillin research and World War II. Dates relating to the Netherlands in World War II, the NG&SF penicillin project, and from the life of Anne Frank's family, are in boldface type

Penicillin research	World War II
1939	
January Florey applies to Medical Research Council for "continuation of work on lytic substances"	
	March Czechoslovakia occupied
	September Germany invades Poland; Britain and France declare war on Germany
1940	
	May 10 **Germans invade the Netherlands; the Dutch surrender five days later**
May 24–25 First mouse experiments performed at Oxford	May 26 British troops begin escape from France via Dunkirk
August First Oxford publication on penicillin as chemotherapeutic agent	**July** **Jews in the Netherlands required to register with Nazis**
1941	
January-June Oxford group successfully treats human patients with penicillin	**February** **Anti-Jewish measures in the Netherlands followed by strike actions; Dutch universities close for duration of the war**
July Florey and Heatley carry *Penicillium* mold to United States; work begins in Peoria, Ill.	
October A. N. Richards meets with selected member of U.S. pharmaceutical industry	
	December 7 – Pearl Harbor attacked by Japanese, United States enters war

matters worse, the weather was unusually cold, and during the notorious Dutch "hunger winter" of 1944–45 more than 16,000 civilians died of starvation.

Remarkably, during these last 16 months of the war, the secret research to produce penicillin, project Bacinol, was conducted in Delft.

A Brief History of NG&SF



F. G. Waller

Jacques C. van Marken, an innovative businessman, founded NG&SF in 1869 to produce baker's yeast. Van Marken believed in basic research and soon established close ties with professors and students at Delft Polytechnic University, where both Martinus Beijerinck and Albert Jan Kluyver served in succession as scientific advisors to the company.

NG&SF expanded and diversified, producing solvents and distilled alcohol in addition to yeast. Between 1920 and 1940, the company acquired over 20 new factories, including production facilities in Belgium, England, Germany, and Portugal. Jenever, or Dutch gin, generated major income, as did other processes for producing glycol and butanol. By the late 1930s, the company headquarters in Delft had laboratories for research and development, a library, instrument makers, glassblowers, and an extensive and well-trained staff of biochemists and microbiologists. van Leeuwen was chief executive officer (CEO), while the director of research and production from 1935 onwards, was F. G. Waller. Waller not only had excellent technical and managerial skills, but he had grown up with the company (his father was CEO before van Leeuwen).

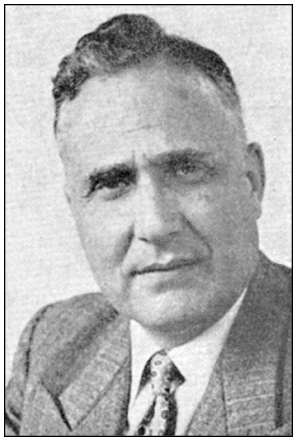
After the German occupation, NG&SF was allowed to function without German management. Its export business was curtailed, so yeast production had to be cut back. Distilled alcohol was deemed a luxury product, with Jenever output going largely to the Wehrmacht. The company had to find new products to fill its fermenters. At the request of the Dutch administration and with the coordination of the Dutch Organization for Nutrition and Food Research (TNO), NG&SF started producing vitamin C, as well as meat and flavor substitutes from yeast and vegetable extracts. The company also increased its production of butanol and butanol derivatives for use as solvents and plasticizers. During the last year of the war, the Wehrmacht commandeered industrial alcohol produced by the company as fuel for German V2 rockets.

Because Delft was not bombed during the war, the NG&SF production site remained intact. Fermentation required specialized skills in microbiology and biochemical engineering, and companies that produced items such as yeast for making bread

were given "essential" status. Between their "special skills" and "essential" status, many of the NG&SF employees were protected from forced labor in Germany. Management had a tradition of benevolence, such as providing employee housing adjacent to the plant, which was extended in 1941 to include a daily subsidized meal for factory personnel. Kluyver later remembered his weekly consulting visit to NG&SF and the warm cup of soup he received there as a rare bit of comfort during the war years.

The one German guard who was assigned to oversee the Delft factory was not skilled in microbiology and could not distinguish the paraphernalia used for routine fermentation processes from that which was used for the clandestine penicillin research project. Moreover, he liked to drink, and management made sure that he was given generous helpings of Jenever.

The Delft Penicillin Research Team



A. P. Struyk

It is not entirely clear how news of penicillin came to Delft in the summer of 1943. Some sources suggest some information was delivered in leaflets, called *De Vliegende Hollander*, or *The Flying Dutchman*, that British fliers dropped. Others say it was announced during Dutch Radio Orange broadcasts from London. However the news about penicillin got through, it was compelling enough to convince Waller that NG&SF should try to make this wonderful new fermentation product.

NG&SF organized a small group of scientists to conduct penicillin research. Waller was pivotal in providing inspiration and leadership throughout. Another key figure was A. P. Struyk, who had completed his Ph.D. in microbial biochemistry under Kluyver. Struyk led the research penicillin team and wrote three of the summary reports preserved in company archives. Other members of the team included A. A. Stheeman, J. Rombouts, and J. M. Klokgieters, head of the fermentation plant. In addition to Kluyver, Andries Querido served as a scientific advisor, despite the fact that he was interned at Westerbork.

The last months of WWII could hardly have been a worse time to launch a research project. Since 1940, materials were in short supply, and the Delft team could not access publications by British and American researchers. Although the Delft scientists were forced to work in secret, they did have one distinct advantage: access to the best fungal culture collection in the world, the Centraalbureau voor Schimmelculture (CBS) in Baarn.

In fact, the first archival evidence of the secret penicillin research consists of dealings with CBS. A letter of 19 January 1944 from CBS director Johanna Westerdijk to J. Rombouts at NG&SF says, "By this mail, we send you the *Penicillium* and

Penicillin research	World War II
1942	
	January 11 Japanese invade Netherlands East Indies
	May Battle of Coral Sea; Corregidor surrenders; Japanese complete conquest of Burma
	July Frank family goes into hiding in Amsterdam
August Several articles in *The Times* (London) focus on penicillin and "miracle cures"	
December Coconut Grove fire in Boston; many patients treated with penicillin made by Merck	
1943	February 2 German forces surrender at Stalingrad
	May Axis resistance in North Africa ends; **Dutch required to surrender their radios**
Summer (exact date unknown) Waller and others at NG&SF learn of penicillin	July Allied invasion of Sicily; Mussolini replaced as Prime Minister of Italy
	September 8 Italy surrenders
December American War Production Board has enlisted 22 major companies to produce penicillin	
1944	
January Rombouts received fungi from Centraalbureau voor Schimmelcultures in Baarn	
March-June Delft team begins penicillin research under leadership of Struyk	

Penicillin research	World War II
June American production of penicillin reaches 100,000 million units per month, enough to treat all the battle casualties of the Allied invasion of Europe	June 6, 1944 D-Day (Normandy invasion)
July 29 **Struyk submits three reports to Waller outlining the development of a bioassay and the isolation and partial purification of an antibacterial substance ("Bacinol") from *Penicillium baculatum***	
	August 4 **Frank family arrested from "Annex" in Amsterdam**
Secret Bacinol research continues; yields increased; Querido obtains Wettstein paper	
	August 25 Paris liberated
	September 3 Brussels liberated
	September 8 First V-2 lands on London
November **Start of Dutch "Hunger Winter"**	**September 17** **Battle of Arnhem**
	December 16 German counteroffensive through the Ardennes (Battle of the Bulge)
1945	**Feb. or March** **Anne Frank dies in Bergen-Belsen**
April **Allied food and medicine drops to Dutch airfields begin despite continued German occupation**	12 April Death of Roosevelt
	30 April Suicide of Hitler
15 May **Stheeman submits 4th report** **16 May Stheeman and Knotnerus submit 5th report; Delft team verifies that Bacinol is penicillin**	**5 May** **Liberation of the Netherlands by Canadian forces** 8 May European war ends (V-E Day)

Aspergillus cultures. Please return the empty blocks, we are not able to get any new blocks at this moment."

Reports in NG&SF Archives Provide Details of Penicillin Project

A. A. Stheeman

The research conducted following the receipt of those cultures is carefully described in six reports preserved in the NG&SF archives. The first three, authored by Struyk and dated 29 July 1944, describe work conducted between March and June 1944. Stheeman wrote the fourth report on 15 May 1945, and coauthored the fifth report with his technician, M. Knotnerus, on 16 May 1945, less than two weeks after VE Day. The sixth and last report, also by Stheeman, is dated 5 July 1945, two months after liberation.

The first in that series of reports, "Bereiding van Bacinol—onderzoek van eenige schimmels op haar vermogen tot vorming van een bacteriostatische stof," or "Production of Bacinol—research on some molds for their potency to produce a bacteriostatic substance," provides a list of the scientific literature consulted, the molds tested, and media that were used for cultivating the molds. The scientists in Delft tested a total of 23 different fungi, including 18 strains of *Penicillium* and 3 of *Aspergillus*. Two additional *Penicillium* strains were isolated in-house from moldy cocoa powder.

This first report cites as scientific sources the prewar publications of Alexander Fleming and H. Raistrick but also included a review article by M. Kiese that appeared in the 7 July 1943 issue of the German journal *Klinische Wochenschrift,* "Chemische Therapie mit Antibakteriellen Stoffen aus Niederen Pilzen under Bakterien," or "Chemical therapy with antibacterial substances from various fungi and bacteria." This article, which contained abstracts of penicillin-related papers that were published by the Oxford Group between 1940–1943, ultimately proved the most useful source of information for the Delft scientists.

The first report also records Delft team attempts to duplicate Fleming's bacteriostatic effect. Fleming had described bacterial lysis, but as Gwyne McFarlane wrote in his 1984 biography of Alexander Fleming, "if the reader had wished to repeat Fleming's observations and experiments he would have found it difficult to discover exactly what he had done." As their test organisms, Struyk and his technician L. P. Lagendijk used *Micrococcus aureus* (Rosenbach) Migul, an old name for *Staphylococcus aureus*, obtained from Kluyver.

The Delft group needed to screen for antibacterial activity. Struyk and Lagendijk seeded plates of peptone agar with a thick suspension of bacteria and added fungal spores. After several days, they hoped to find "halos" of bacterial lysis resembling

those reported by Fleming, but found no evidence that bacterial growth was inhibited. Next they devised an agar block method. The mold was grown on agar, and then a small plug of agar and mold was placed onto another plate already inoculated with bacteria. Using this approach, clear areas were observed around the agar plugs of seven of the tested strains. The one with the greatest antibacterial activity was coded P-6, *Penicillium baculatum* Westling.

A Pencillin-Producing Strain Is Identified and Its Products Extracted and Tested

In his second report, Struyk describes how flasks containing NG&SF's Liquitex bran-and-malt medium were inoculated with *P. baculatum*, grown for 5 days at 26°C, and shaken once a day. The antibacterial substance produced by P-6 was soluble in acetone and alcohol, which facilitated extraction from the broth, and, when mixed with water, was also resistant to boiling. Struyk named the antibacterial substance "Bacinol" after its producing species, *Penicillium baculatum*.

That name was both scientifically and politically prudent. Without an authentic standard of penicillin, it was not possible to tell if "Bacinol" was actually penicillin—as they hoped—or whether it was some other antibacterial substance. The code name also helped ensure that the Germans would remain unaware of the true nature of the clandestine research.

Fleming and the Raistrick group extracted penicillin into ether after acidifying the culture filtrate. However, when the ether evaporated, the antibacterial activity disappeared. Later, Abraham and Chain showed that if the ether was back-extracted into a buffer near neutral pH, antibacterial activity was retained. The Delft group followed the Fleming-Raistrick protocol and similarly lost almost all activity. They next tried Abraham and Chain's methods, which were described in Kiese's article, washing the ether extract with a phosphate buffer of about pH 6.5. Thus, by June 1944, they produced a small amount of a gold-brown extract, estimated to be 50% Bacinol.

Rombouts and his assistant Ans Addeson tested this material on infected rabbits and mice. Animals treated with Bacinol recovered from infection, while untreated animals died, indicating that Bacinol was effective in vivo and relatively nontoxic.

Delft Team Quickly Scales Up Production of Bacinol

Convinced that they were on the right track, the members of the Delft team quickly considered how to scale up Bacinol production. They soon decided to use milk bottles because, in spite of wartime shortages, they were available and relatively easy to sterilize. Struyk requested Klokgieters to empty a room in the pilot plant for "hundreds of milk bottles" (Fig. 1). To monitor production, members of the Delft team designed a quantitative bioassay in which *Micrococcus aureus* "strain 6" served as an indicator species and the concentration of Bacinol was expressed in Delftsche Eenheden (D.E.'s or "Delft Units"). DE was defined as "the amount of bacteriostatic substance which can just completely suppress the growth of the test organism *Micrococcus aureus* strain 6 in 1 ml of peptone water at 37°C."

Sometime during the summer of 1944, the

Penicillin research	World War II
	3 June Otto Frank returns to Amsterdam from Auschwitz
5 July Stheeman submits last of wartime penicillin reports	
	6 August Atomic bomb dropped on Hiroshima
	15 August Japan agrees to surrender (V-J Day)
November **Two young women, critically ill with *Staphylococcus* infections, are successfully treated in Bethel Hospital, Delft, using NG&SF penicillin**	20 November Nuremberg tribunal begins
December Nobel Prize awarded to Chain, Fleming, and Florey	
1946 **1 January** **K. Scheurkogel is appointed co-coordinator of a NG&SF department devoted solely to penicillin production**	
1947 **Waller buys stainless steel company to build new fermenters**	June U.S. Secretary of State George Marshall announces plan for massive aid to Europe
1948 **NG&SF meeting all Dutch requirements for penicillin**	
1949 **NG&SF begins exporting penicillin**	

Delft scientists received help from A. Querido, a Jewish physician and NG&SF advisor who had been interned at Westerbork with his wife and young son and was under threat of being transfered to Theresienstadt, an extermination camp. Company management declared him an "essential worker" and, once a month, he was allowed to leave the camp to attend meetings at NG&SF in Delft. While changing trains on one of these trips, he met a colleague from the University of Amsterdam who told Querido that a recent visitor from neutral Portugal had brought a Swiss medical journal with an article by A. Wettstein on penicillin. Querido brought the borrowed publication to NG&SF for copying, providing further corroboration that the Delft team was on the right track.

Thus heartened and, despite the deteriorating conditions in the Netherlands during the hunger winter of 1944–1945, the team in Delft continued to make progress. The fourth report describes buffers with which to extract ether from Bacinol; trials to improve growth conditions for *Penicillium* by substituting Jena and Roux bottles for milk bottles; and the search for improved "mash" for growth media, using beet pulps and grain mixes.

Near the end of the war in April 1945, the Germans allowed the Allies to drop food and medical supplies, including penicillin, to the starving Dutch. From one such drop, E. Verschuyl, who served as the NG&SF company doctor, managed to obtain a few penicillin ampoules that were manufactured by Charles Pfizer & Company and supplied by Upjohn of Kalamazoo, Mich. Scientists at NG&SF immediately began comparing the contents of those ampoules to Bacinol.

When the war in Europe ended a few weeks later, the liberation was made even sweeter for the members of the Delft team when they verified that "Bacinol" was indeed penicillin. The last of the relevant archival documents summarizes the work on comparing the antibacterial substance from *P. baculatum* with other sources of authentic penicillin. The Delft team scientists concluded that the only detectable difference was that Bacinol was yellowish in color, whereas the American product was white. As the Delft team believed all along, Bacinol was penicillin.

The Delft team continued its efforts to scale up production. After several unsuccessful attempts, its members developed a key double-steam sealing and sterilization process. For several years, NG&SF continued using its P-6 strain of *P. baculatum*, noting that it compared favorably to other penicillin-producing strains of *P. notatum* and *P. chrysogenum* that were obtained from CBS. Later, in a taxonomic study, Charles Thom concluded that another *P. baculatum* producer strain was probably *P. chrysogenum*, but he never examined the NG&SF production strain. Eventually, NG&SF, like all penicillin manufacturers, switched to using a higher-yielding derivative of the Peoria strain NRRL 1951. It would be interesting to go back and conduct a molecular taxonomic analysis of the original P-6 production strain of *P. baculatum*.

FIGURE 1

Milk bottles containing *Penicillium baculatum*.

Epilogue

Why isn't this remarkable story about the Bacinol project better known? With the exception of Gladys Hobby's 1985 book *Penicillin, Meeting the Challenge*, which devotes two and a half pages to "the Yeast Factory in Holland," and a few Gist-brocades company publications, little has been written about this extraordinary Dutch achievement until the recent research of Marlene Burns.

Part of the reason may be the inherent reserve of the Dutch people. The group at NG&SF had not discovered penicillin but had identified a production strain, created a bioassay, developed media, perfected a purification protocol, and built and refined submerged fermentation tanks—all noteworthy achievements reflecting exceptional technical prowess.

Perhaps the main reason for the lack of fanfare was the reality of life in postwar Europe, with acute shortages, rampant malnutrition, and widespread disease. In most of Europe, demand for penicillin far outstripped the supply, and it became a valuable black market commodity. However, the Netherlands quickly produced sufficient supplies of penicillin. By 1946, NG&SF was supplying all the penicillin needed by Dutch hospitals; by 1948, it was supplying all the penicillin needed for the entire country; and by 1949 it began exporting penicillin. Eventually, NG&SF became one of the world's largest penicillin producers. The circumspect and ethical management of NG&SF felt no need to draw attenton to themselves in those difficult times. It was more than sufficient reward to save lives and to help the Dutch economy.

In 1950, NG&SF was awarded royal status (Koninklijke); in 1967 KNG&SF merged with Pharmaceutische Fabrieken v/h Brocades Stheeman & Pharmacia to become Gist-Brocades NV; in 1998, Gist-Brocades became part of DSM, a Dutch chemical company.

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