In November 1945 the recovery of Maria Gene, a patient in Delft’s Bethel Hospital signalled the success of the secret production of penicillin at the NV Nederlandsche Gist- en Spiritusfabriek (NG&SF) in Delft, The Netherlands, during the Second World War, whilst under occupation by Nazi Germany.
Penicillin at NG&SF 1940 - 1950

NG&SF:
Cut off from the outside world

- Because of Occupation

Cut off from all information

- From 1943 to 1946 there was an allied embargo on publications concerning the production and chemistry of penicillin

   North Africa Campaign

No information on the development of Penicillin allowed outside Britain and the USA
‘this penicillin is as good as anything here (UK) or in America’.
Alexander Fleming, November 1946
How, therefore, was the development of Bacinol, Dutch penicillin, possible?

- How was it kept secret?
- Who were the researchers?
- How was it produced?
Penicillin at NG&SF 1940 - 1950

Introduction

- NG&SF: The Gist
- Experience under Occupation during World War II
- The Delft Team
- Penicillin on a Large Scale
- Concluding Remarks
Penicillin at NG&SF 1940 - 1950

Pre-war NG&SF

- In contrast to general industry NG&SF was buoyant
- 1920 - 1940 was a period of industrial expansion and R&D
- Market leader with “Koningsgist”
  Other products Butanol, Acetone, Ether and Engedura (dried yeast)
- Prestigious reputation as fermentation authority
- Subsidiaries in Brugge, Monheim, London, Portugal.

M. Burns PhD
The Gist in the war years

- NG&SF fermentation reputation allowed them to stay open
- *Wet Uitzonderingsgevallen* allowed producers of essentials to stay open
  - Bread
- Personnel received “required worker” status
- Fermentation around the clock process – protected workers came and went even during curfew
- In 1942 NG&SF accounts show a relatively high Share Dividend of 15%
As the war progressed

- Production reduced - cut off from affiliates and restricted to local market
- Raw materials only from Rijksbureaus - rationed
- Deportation of NG&SF workers – Monheim / ‘onderduikers’
- Food shortages - factory meals - zuurkool en graanpap slechts 5 cent per bord
  - Encouraged - ‘grow your own’ in factory allotments
Conclusion

- NG&SF wartime experience:
  - Management stayed in place and gained experience
  - Lack of materials stimulated ingenuity in R&D
    Gistex – Aromex
  - Lack of production gave rise to opportunity for R&D
    Vitamin C – Shell and Naarden

*New processes which would stand us in good stead in the production of penicillin.*

F.G. Waller, Jnr.
“They were exciting days for us and not only because of the advance of the allies”
F.G. Waller, Jr.

Claims news of allied “wonder drug - penicillin” from:

- Underground Radio Oranje? BBC?
- “Vliegende Hollander” (Flying Dutchman) leaflets?
- “De Vervelwind” (The Whirlwind)?
NG&SF R&D Report 412 March-June 1944

NG&SF started with:
- Clutterbuck et al (1932) *Biochem. J.*

The articles by Fleming and Clutterbuck were inconclusive but easily available from the pre war literature.

Source: Dr. A.P. Struyk.
Dr. A.P. Struyk

Penicillin at NG&SF 1940 - 1950

M. Burns PhD
Starting Position

Penicillin at NG&SF 1940 - 1950

- Waksman (1940) *Chronica Botanica*
- Penau, Lavaditi and Hageman (1943) *Bull de la Societe de Chemie Biol.*
- Vonkennel, Kemmig and Lembke (1943) *Klinisch Wochen.*
- Kiese (August 1943) *Klinisch Wochen.*

*Manfred Kiese, University of Berlin. Listed 61 footnotes of sources between 1923 and 1943.*

*Kiese’s publication was of great value regarding the research of Florey and Chain.*

M. Burns PhD
Prof. A. J. Kluyver

Penicillin at NG&SF 1940 - 1950

M. Burns PhD
In the Kluver Archive there are photocopies of various wartime publications regarding penicillin and which include


and


They are marked by the annotation ‘Bibliotheek DBM’, ‘Delft, Brugge, Monnheim’, the latter two being subsidiaries of NG&SF. Brugge, Belgium, and Monheim, Germany.

Inter-Library Loan. Jan de Flines
Dr. A.P. Struyk received 21 fungal strains from the CBS (Baarn)

- 18 penicillium strains
- 3 aspergillus
- NG&SF supplied 2 fungal strains from cacao powder

In the CBS archives there is no record of a special request from NG&SF and the director Prof. J. Westerdijk may have secretly collaborated with NG&SF. Contact: JR Rombouts.
Prof. Dr. Joanna Westerdijk

Penicillin at NG&SF 1940 - 1950

M. Burns PhD
To culture the different strains, Struyk used an existing NG&SF bran and malt growth medium, *Liquitex*.

To evaluate the production of an antibacterial substance Struyk developed a zone of inhibition agar block test with *Micrococcus aureus*.

Using this method the activity of the strains could be compared.

To monitor production the Delft Unit of antibacterial activity was defined.

*Florey and Chain had already defined the Oxford Unit of antibacterial activity for precisely the same purpose.*
Fleming’s Contaminated Petri Dish
In July 1944 Struyk reported to Waller, Stheeman and Rombouts:

- 5 penicillium, 1 aspergillus and 1 cacao strain produce an antibacterial substance

- *Penicillium baculatum* Westling gives the highest yield

- Struyk names the substance produced – Bacinol

- It is soluble in alcohol and acetone and is resistant to boiling

- Bacinol has the same antibacterial and physical properties as penicillin (as described by Fleming)
Stheeman, Knoterus and Mathu develop an extraction method

Ether is chosen as extraction medium
Dr. A.A. Stheeman

Penicillin at NG&SF 1940 - 1950
Recovery

In June 1944 NG&SF produces first small amount of gold coloured powder - 50% pure

Milk bottles the ‘natural fermentor’ increase yield
The effect of ‘Chance’: July/August 1944

- Wettstein (1944) Schweizerische Medizinische Wochenschrift entitled ‘Penicillin’

This journal was secretly provided by Prof. Querido via

- Barneveld, Camp Westerbork, neutral Portugal and Amsterdam Central Station
Dr. Andries Querido

Penicillin at NG&SF 1940 - 1950

1990 1947

M. Burns PhD
August 1943 Kiese’s publication based on 61 sources; June 1944 Wettstein, 159.

Wettstein’s article clearly illustrated the methodology used by Florey and Chain at Oxford.

In fact, this whole issue of SMW is given over to the development of Penicillin and offers the most up-to-date information of what was happening in both the UK and USA.

It offered the last building block for the Delft Team, with this information they knew they were on the right track. Kluyver, photocopy number 13.
Exciting days for us and not just because of the advance of the Allies.... By around Dolle Dinsdag we had a small amount of a substance which we hoped, and later to our joy proved to be, penicillin.

F.G. Waller, Jnr

Dolle Dinsdag – 5 September 1944
BUT Arnhem failed

The South of the Netherlands was liberated
The North and Western provinces remained occupied

M. Burns PhD
The Hunger Winter
Toxicity and Efficacy

Between July 1944 and March 1945

Rombouts and Ans Addeson demonstrate that Bacinol is safe and effective in infected rabbits and mice
On 28 April 1945 there are Allied food drops at Ypenburg, Duindigt, Valkenburg and Waalhaven.

Struyk obtains samples of Pfizer/Upjohn penicillin and in July 1945 reports:

- Bacinol = Penicillin
- 10 Delft units equal 1 Oxford unit

At the end of this report the name Bacinol is replaced by Penicillin
In U.S.
Merck, Squibb, Pfizer, Winthrop and Abbot combine forces under the U.S. War Production Board (WPB)

In U.K.
Boots, British Drug Houses, Burroughs Welcome, May and Baker and ICI combine forces as the Therapeutic Research Corporation of Great Britain

In Occupied Netherlands, NG&SF isolated.
In need of investment. In need of raw materials.
Penicillin at NG&SF 1940 - 1950

- Open surface replaced by deep fermentation technology – Kluyver 1930s

- Waller releases order for a new fermentor in pilot plant F3

- Department established
- K. Scheurkogel employed as penicillin co-ordinator
- R.A. Jellema head Penicillin Department

- 6 months after the end of the war, October 1945
  15 hectolitre tank yields 15g penicillin
Scale up

Penicillin at NG&SF 1940 - 1950
Clinical Experience

In the Bethel Hospital in Delft Dr. Evert Verschuyl treats two young women dying of septicaemia with NG&SF penicillin.

Patient 1 - 28 years, Maria Gene, temperature persistently 39/40°C. Nov 15 receives 50,000 units penicillin - temperature returns to normal - patient discharged Nov 29 – 14 days.

Patient 2 - 18 years, temperature 40.8°C. Nov 26, 27 and 28 receives 50,000, 100,000 and 150,000 units penicillin - patient discharged Dec 6 – 10 days.
January 1946

Newly formed Government Commission for Antibiotic Medicines

Overseen by the State but not funded by the State

‘Big Science’

‘Teamwork’
Position 1945/46

- 1945 U.S. and U.K. producing as much Penicillin as possible but not enough to fill demand
- NG&SF no need to wait for licence
- NG&SF own strain
- NG&SF own production technique
- NG&SF own development
- A ‘will’ to succeed
Penicillin at NG&SF 1940 - 1950

- Freeze drying techniques learned from Blood Transfusion Service (CLB). Freeze drying – the breakthrough to stability

- Filter system invented – the Hornex, a counter current

- Sterile conditions met with ‘Double Steam Sealing’

- Waller purchases Leidse Machine Fabriek (LAF) to obtain exclusive rights to stainless steel

- As the Allies lift the embargo on penicillin data

NG&SF ‘critical step’ from fermentation to pharmaceutics

M. Burns PhD
15 May 1946
The first industrial fermentation
1.5 hectolitre *Ensinkkettel*

Followed by upscaling to
15, 60 and 300 hectolitres

Johan van den Berg, *Bedrijfsassistent*
In November 1946 Kluyver met Alexander Fleming at Pasteur Institute in Paris.

He had taken with him a sample of NG&SF penicillin which he asked Fleming to have analysed.

Fleming did this through Glaxo Laboratories and concluded that:

‘this penicillin is as good as anything here or in America’.
Medical Brains Trust:
  Kluyver (Chair)
  Waller and Querido
  Jacob Mulder, W.R.O. Goslings, Leiden
  L.E. Den Dooren de Jong, Delft

Digesta Antibiotica

Johanna Westerdijk.
August 1946

Seven hospitals involved:
Academische Ziekenhuis in Leiden, Utrecht and Groningen;
Johannes de Deo, The Hague;
Wilhelmina Gasthuis and Binnen Gasthuis, Amsterdam; and
St Jacobus Stichting, Wassenaar.
With the Gemeente Apotheek, The Hague
By 1946 NG&SF was supplying all the Penicillin needed by Dutch hospitals.

By 1948 NG&SF was supplying all Penicillin requirements for the whole of the Netherlands.

In 1949 NG&SF started exporting Penicillin.

In 1950 it received ‘Koninklijke’ (Royal) status.

Only 50 years from the end of the Second World War, Gist Brocades was one of the world’s largest producers of Penicillin.

March 2005, 60 years later and production in Delft stops.

M. Burns PhD
How was Bacinol kept a secret?

- Germany had little fermentation experience and the German pharmaceutical industry was focussed on synthesis.
- Fermentation is a 24 hour process and the Germans were used to seeing workers coming and going even through curfew.
- Nothing unusual about milk bottles ‘growing stuff’. (de Horn)
- I just don’t think it was expected at a yeast factory in Delft.
NG&SF – Critical Success Factors

Penicillin at NG&SF 1940 - 1950

- NG&SF was not a pharmaceutical company. They did not need to invent the therapeutic concept of the “wonder drug”, penicillin.

- The development of penicillin was a technical problem that required in-depth knowledge of microbiology, fermentation and recovery.

- NG&SF had unique access to TU Delft where Prof. Kluyver was a world authority. NG&SF employed many former his students. A close relationship.

M. Burns PhD
Penicillin at NG&SF 1940 - 1950

- F. G. Waller was a determined, inspirational leader with an excellent technical grasp and an affinity and flair for improvisation

- The Delft team was small, cohesive with no bureaucracy and short lines of communication

- The Delft Team was determined to succeed

- Serendipity - Professor Querido

“Producing Bacinol was not a duty… it was a pleasure… we wanted to succeed”

de Horn

M. Burns PhD
Alexander Fleming’s Laboratory

Penicillin at NG&SF 1940 - 1950
The Delft Team

Penicillin at NG&SF 1940 - 1950

**Leader:** F.G. Waller (NG&SF Deputy Director)

**Microbiology:** Struyk, Stheeman, Knoteerus, Lagendijk, Mathu, Rombouts and Addeson

**Fermentation:** Klokgieters, Verkennis

**Upscaling:** Jongbloed, van den Berg, Enzenga, de Horn, ter Horst, Mensinga, Mosterd, Saltet; Berends, Scheurkogel and Jellema

**Clinical:** Dr. Verschuyl

**Advisors:** W.H. van Leeuven (NG&SF President), H.F. Waller (NG&SF Deputy Director), Profs. Kluyver (TH, Microbiologist), Westerdijk (CBS), Querido with Mulder and Goslings (Leiden Univ., Physicians).

*M. Burns PhD*