

Penicillin and Serendipity

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International Day of Fighting Infection, 23 April

Penicillin: a captivating story



Penicillin and Serendipity

SERENDIPITY

“The faculty of making happy and unexpected discoveries by accident”

-from Horace Walpole (1754) after *The Princes of Serendip*
(Oxford Concise Dictionary)

PENICILLIN

- happy and unexpected discovery?
- an accident?

Was the penicillin story a series of lucky events?

The 'Eureka' moment and beyond.....?

- The result of a surprise 'happy and unexpected discovery' by accident? ...or...

The result of painstaking research, study, experiment and observation with foresight and belief?

- A discovery by a maverick individual with a singular vision who discovers what they have been seeking (against disbelief, ridicule, adversity) ...or...

The result of dedicated and focussed teams working towards clear targets and objectives

"It is the individual human, working out his own design of life who gives the world what progress it has made"

Alexander Fleming

“Science is the graveyard of ideas” Pasteur

leading to

Something with limited application, known only to few and can't be developed, made available or used

...or...

Something needed, developed, usable, known and available

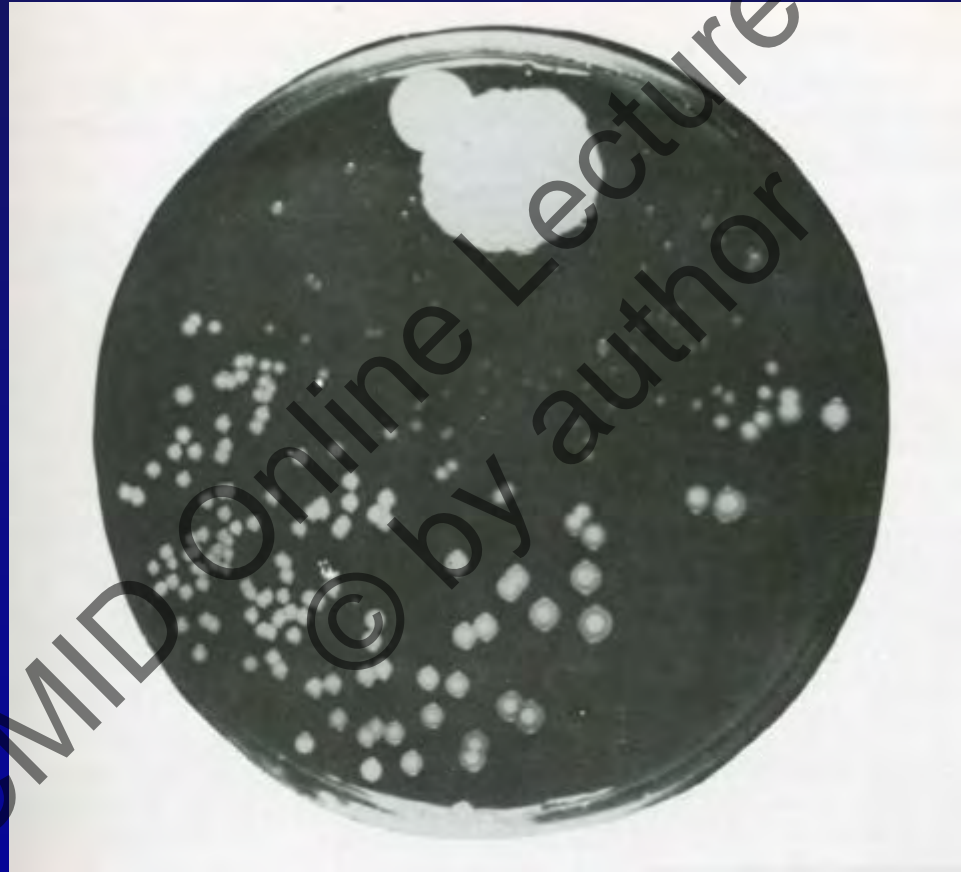
It is only those with a value and use which we are able to look back on today in the context of their role in the evolutionary progression of technical achievement.

Mould juice to 'penicillin': serendipity?

- 47year old **Alexander Fleming** ('Flem') appointed Prof Bacteriology at St Mary's Hospital Medical School Saturday 1 September 1928
- returned from holiday on Monday 3 September & examined discarded petri dishes, left for a few weeks as was his habit, with cultures of *Staphylococcus aureus* before destroying them
- interrupted by Merlin Pryce (ex colleague) who encouraged him to continue to look at them.....

“That’s funny!”

'That's funny!'



“Happy and unexpected discovery by accident”?

The right mould on the right plate at the right time?

And seen by the right man?

Old petri dishes: untidy workmanship?

Contamination : a lucky breeze?

S. aureus and *P. notatum*: coincidental lucky partners?

Happy and unexpected discovery by accident?

- Fleming prepared the plates as he was working on a Chapter on staphylococci for 'A System of Bacteriology' for the MRC
- He was looking at colour variation over time at different temperatures

“ Now had I been intensely interested in staphylococcal variation and not antibacterial substances I would have cast out the plate...” Fleming

- Pryce thought lysis was due to acids...but commented *“That’s how you discovered lysozyme”*

“Chance events favour only the prepared mind” Pasteur

Happy and unexpected discovery by accident?

Fleming's 'prepared mind'?

- “ My important part ...was that I saw something unusual and appreciated something of its importance so that I set to work on it. I have no doubt that the same phenomenon had been presented by accident to other bacteriologists, but they were not interested and so the chance was missed....”

“Purge me with Hyssop...and I shall be clean”

- Hyssop for ‘all manner of leprosy and scall’
 - ◆ *P.notatum* first described from hyssop plant,1911
- Thomas Phayre, 1545, mushroom from elder tree
- Lancet 1852: troublesome boils: yeast extract
- Lister (1870’s): fungi prevent bacterial growth
 - ◆ Lister used *P.glaucum* in gluteal abscesses
- Sanderson, 1871; Tyndall,1876; Gosio, 1896; Duchesne, 1897; Sturli, 1908; Alsberg & Black, 1913; Gratia & Dath, 1924: penicillium extracts
- 1913, *P.glaucum* (Cambridge) taken for a ‘salve for gatherings’

S. Selwyn, JAC, 5, 249-255, 1979

S. Selwyn, The b-lactam antibiotics,1980

Flemings prepared mind

St Mary's Innoculation Department 1906-1955 under Sir Almroth Wright FRS

'after food & shelter the most urgent need for a civilised community is for medical research'

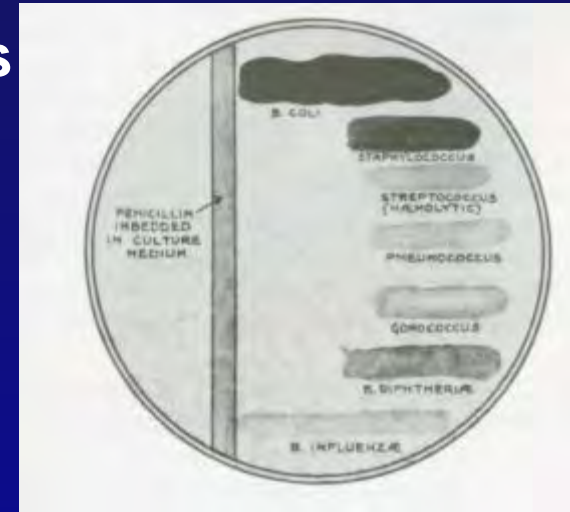
Faith in the principle of vaccination: Opsonin

Fleming

- worked on Opsonic Index and vaccine therapy
- authored papers ('Therapeutic Immunisation' Lancet) from 1907
- administered Salvarsan as a 'pox doctor' from 1909
- studied wound infection (*C.welchii* from clothes) in 1914 WW1
- saw that antiseptics compromised healing
- isolated *H.influenzae* B in the 1918 flu epidemic
- discovered lysozyme (1921) from his own nasal mucus, and which inhibited *Micrococcus lysodeikticus*

But what next?

- He saved the plate; sub-cultured the mould
- He gave it a name: penicillin
- He studied and published its properties
 - ◆ gutter plates (mould 5 days room temp.)
 - ◆ streaked with test bacteria (37°C)
 - ◆ ‘bacteriolytic for staphylococci’
 - ◆ staphs, streps, gonococcus
 - ◆ non toxic in animals: but short-lived activity
- He tested it in people (Craddock ate it first)
 - ◆ sinusitis (Craddock): little effect
 - ◆ amputation stump: irrigated but patient died



But what next?

- He told people about it
 - ◆ MRC 1929: 'A medium for the isolation of Pfeiffers bacillus' (selecting *H.influenzae* for 'anti-influenza' vaccine): no questions or interest
- He published
 - ◆ British Journal of Experimental Pathology: June 1929: 'On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of *B.influenzae*'
'it may be an efficient antiseptic for application to or injection into areas infected with penicillin-sensitive microbes'
- He gave people cultures
 - ◆ Lister Institute; William Dunn School, Oxford;
Sheffield University Medical School

The river dries up?

Fleming continued to treat with penicillin ('juice')

- sinusitis, septic wounds, conjunctivitis (first success 1933)
- George Payne (ex St. Mary's) used it in Sheffield in eye infections in babies and successfully treated an adult with a pneumococcal eye infection: but failed to publish
 - ◆ Howard Florey (Prof. of Pathology at Sheffield) did not show interest....
- Harold Raistrick (LSHTM) could not isolate penicillin

Oxford, August 1940

PENICILLIN AS A CHEMOTHERAPEUTIC
AGENT

BY

E. CHAIN, PH.D. CAMB.

H. W. FLOREY,
M.B. ADELAIDE,

A. D. GARDNER,
D.M. OXFORD, F.R.C.S.

N. G. HEATLEY, PH.D. CAMB.

M. A. JENNINGS,
B.M. OXFORD,

J. ORR-EWING,
B.M. OXFORD,

A. G. SANDERS,
M.B. LONDON.

(From the Sir William Dunn School of Pathology, Oxford)

IN recent years interest in chemotherapeutic effects has been almost exclusively focused on the sulphonamides and their derivatives. There are, however, other possibilities, notably those connected with naturally occurring substances. It has been known for a long time that a number of bacteria and moulds inhibit the growth of pathogenic micro-organisms. Little, however, has been done to purify or to determine the properties of any of these substances. The antibacterial substances produced by *Pseudomonas pyocyanea* have been investigated in some detail, but without the isolation of any purified product of therapeutic value.

And the 'late' Prof. Fleming pays a visit.....

The missing link

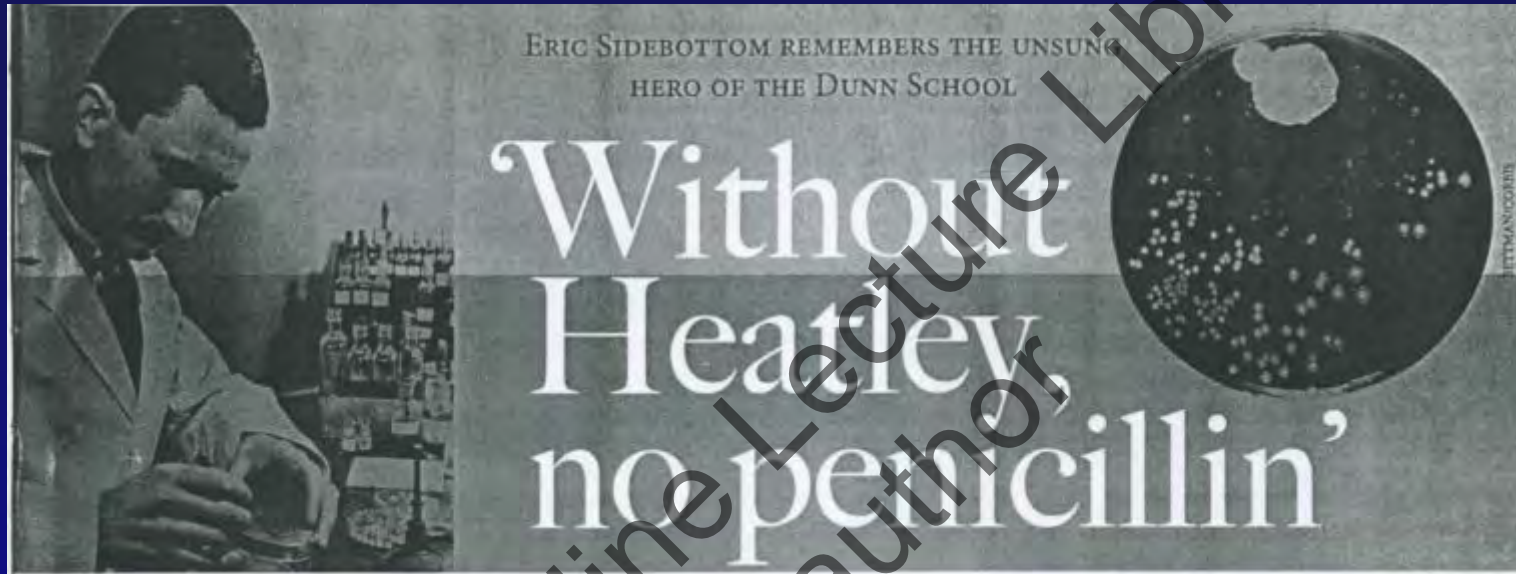
- Florey, 1935; Chair of Pathology: **lysozyme**
- Ernst Chain, 1937; nature and MOA of **lysozyme/lysis**
 - ◆ antibiosis. *B. subtilis*, *B. pyocyaneus*, *P.notatum*
 - ◆ **bacteriololysis against pathogenic bacteria**
- Fleming had described the **lytic** properties of penicillin
- *P.notatum* culture already at Dunn school

The Oxford Group : the value of a team?



- **Howard Florey: Prof Pathology**
- **Ernst Chain: purification/biochemistry**
- **Edward Abraham: purification/biochemistry**
- **Norman Heatley: production/biochemistry**
- **A.D.Garder: potency/microbiology**
- **Jean Orr-Ewing: potency/microbiology**
- **Gordon Sanders: pathologist**
- **Margaret Jennings: biology/toxicology**

But no penicillin without Heatley?



- Penicillin had been lost in attempts to retrieve it from ether.
- Heatley used 'back titration'
 - ◆ mould extract down and ether up a tube: penicillin collected at the top and then back again into water.....
 - ◆ stored in fridge (freeze-drying later)

Proof of concept : 1940

- **Mice:**
 - ◆ non-toxic, destroyed by gastric acid, in urine
 - ◆ lethal strep infection cured by injection
- **Man:**
 - ◆ tested for toxicity on a dying 50 year woman
 - ◆ Albert Alexander : severe staph/strep septicaemia; improved at 5 days but recycled penicillin ran out
 - ◆ children and more adults cured: Lancet 1941
 - ◆ Fleming cured Harry Lambert of meningitis (with Floreys help) in 1942

Cooperation

- **Therapeutic Research Corporation, 1941 and General Penicillin Committee**
- **Boots, M & B, BDH, Glaxo, Burroughs Wellcome**
- **Followed by ICI, Distillers, Allen and Hanbury**
- **Trays, bottles.....**

1940's trans-atlantic collaboration and production



Plate XXXII a (above) General view of the operating floor of a fermenter hall; b (below) Photomicrograph of *Penicillium* culture (from Lawson, 1944).

A RACE AGAINST DEATH!

The faster this building is completed... the quicker our wounded men get **Penicillin**

THE NEW LANCETON DRUGS

Get this for EVERYTHING you get!

An exhortation to build a penicillin factory to aid the war effort. (Alexander Fleming Laboratory Museum, St Mary's NHS Trust)

submerged culture and corn-steep liquor

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1950s – The penicillin ‘family’

penicillin G (benzyl) - injection

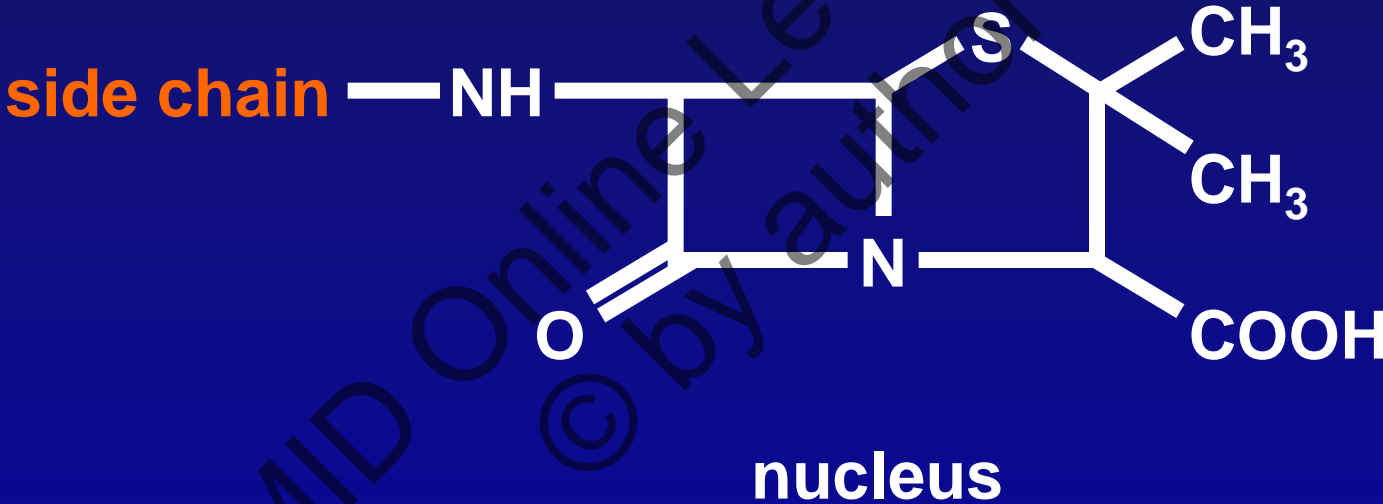
penicillin V (phenoxymethyl) – tablet

(Behrens, 1948 (from fermentation + phenoxyacetic acid))

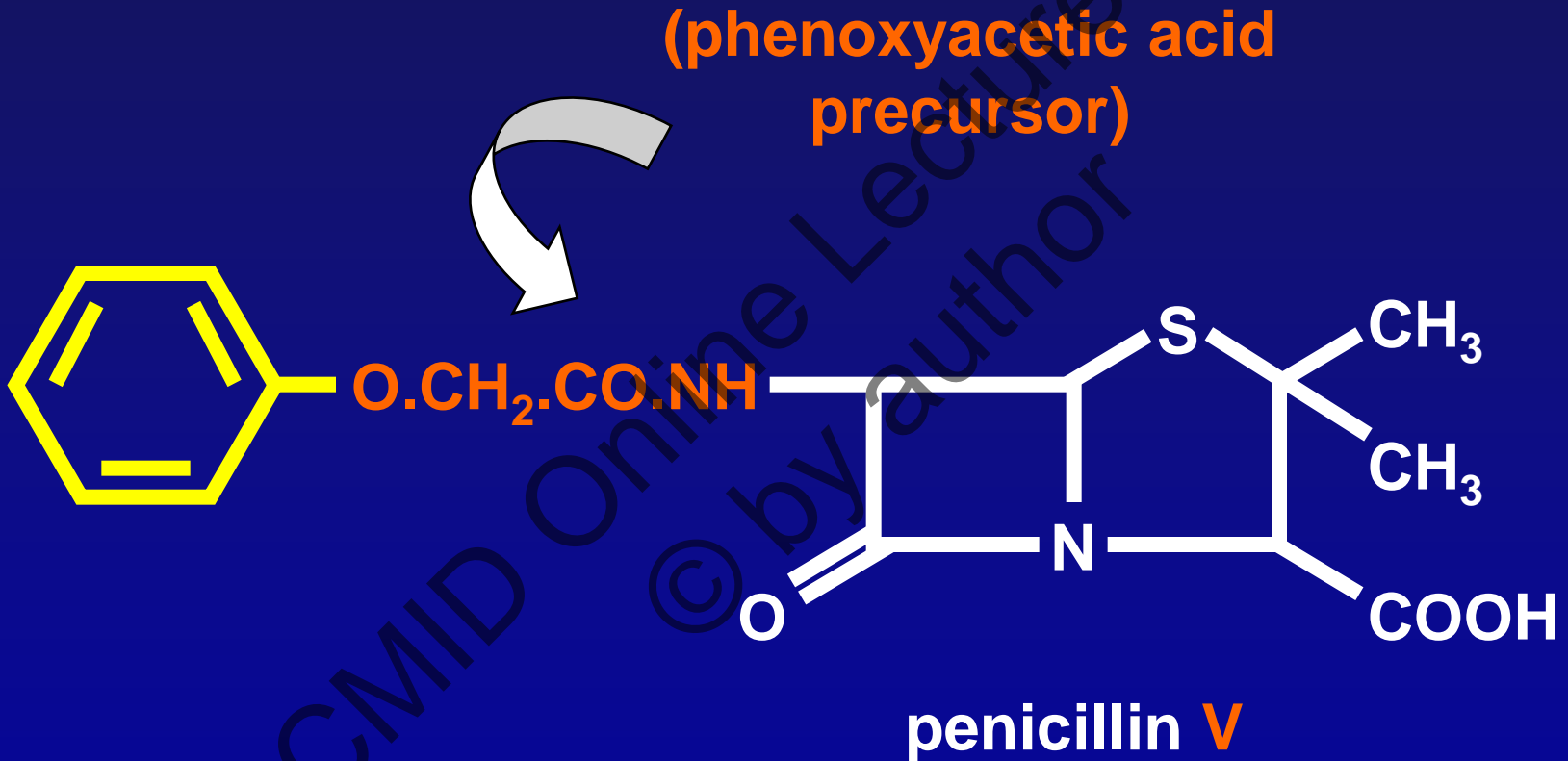
**Only effective/active against a narrow range
of bacteria types**

Streptococcus, Staphylococcus, Gonococcus

Penicillin structure



Penicillin fermentation



From Pills to Penicillin

- 1938: Beecham Macleans Holdings formed as a subsidiary of Beecham Pills Ltd (Toothpaste, Eno, Brylcream, Lucozade)
- 1942: formed a research board to look into 'ethical products'
 - ◆ 'special consideration to biological and bacteriological research'
- employed scientific advisors (eg Prof E.C.Dodds)
- 1945: bought a research site (country house) and formed Beecham Research Laboratories Ltd

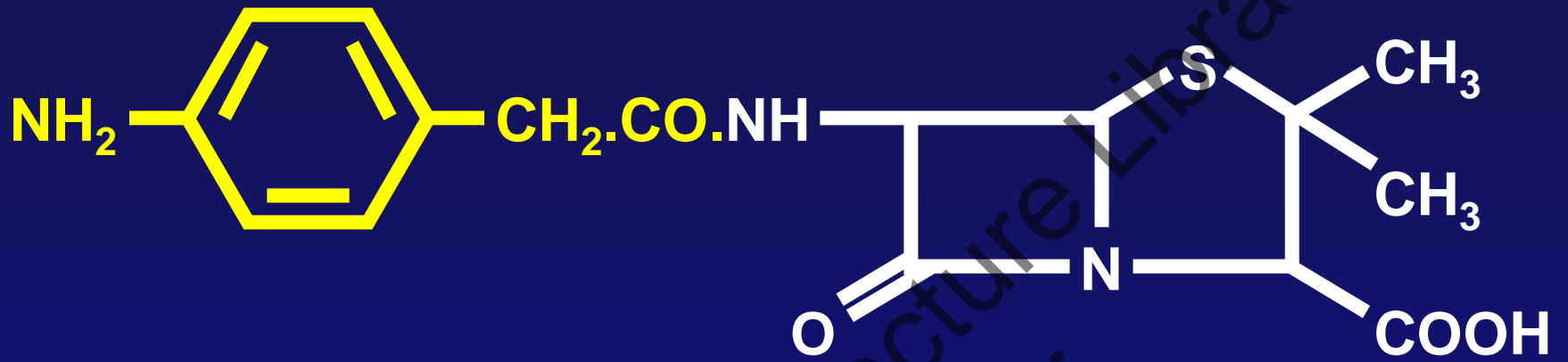


From Pills to Penicillin

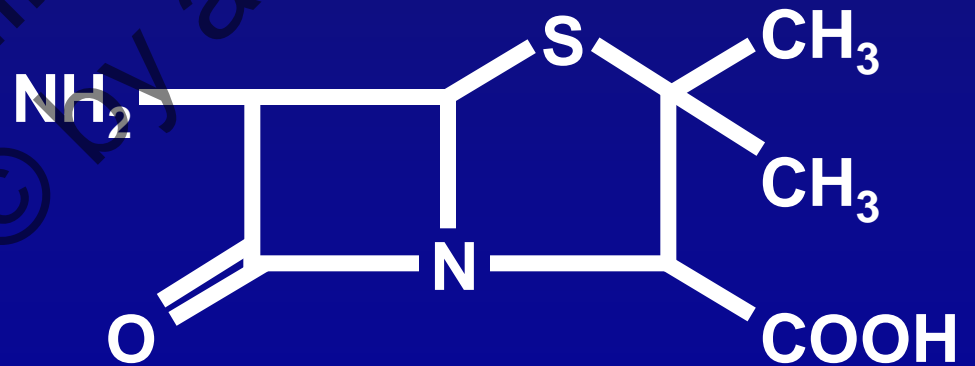
- BRL looked into
 - ◆ methionine (cirrhosis)
 - ◆ anti-TB drugs (proved toxic)
 - ◆ penicillin (pastilles and oral formulations pre 1952/Pen V)
- Proposal to set up (with Bristol-Myers) a general fermentation plant (1952-4) for amino-acids and penicillin G
 - ◆ Consulted with Ernst Chain, who was convinced that there was more to discover in this area: engaged as a consultant

From Pills to Penicillin

- 1955: Beecham decided to enter antibiotic field to produce penicillin at a competitive price to Glaxo
 - ◆ (Glaxo and Distillers were producing based on Pfizer and B-M methods)
- Chain was actioned 'to prepare a memorandum on current oral preparations, on chemically modified penicillins and to arrange and attend a discussion on the purely chemical problems at Brockham Park'
- Three microbiologists /biochemists were recruited to commence work in Rome (at Chain's facility) {George Rolinson, Ralph Batchelor}
- Para-aminobenzyl penicillin produced in Rome and in a small pilot plant commissioned at Brockham Park

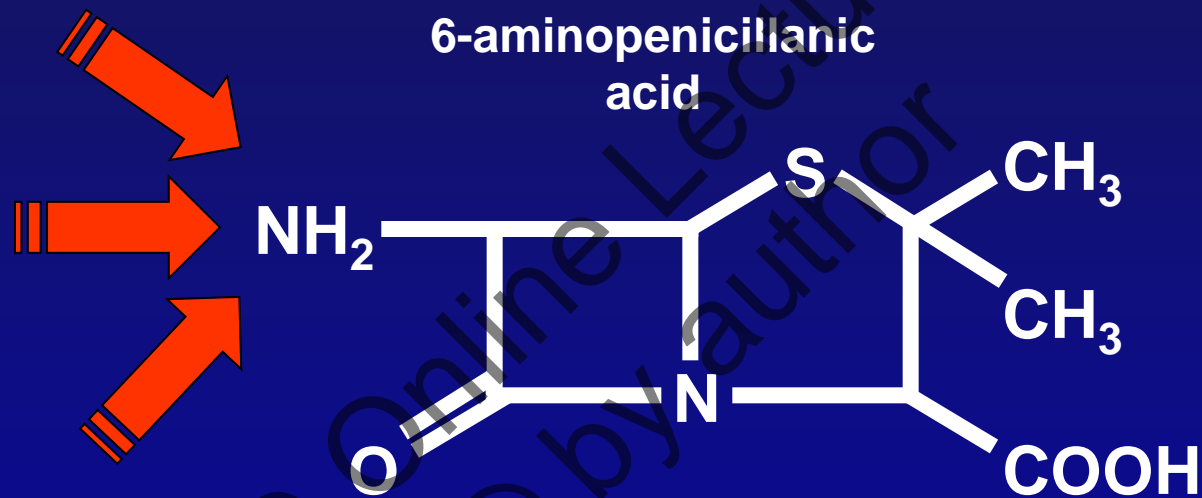


p-aminobenzylpenicillin



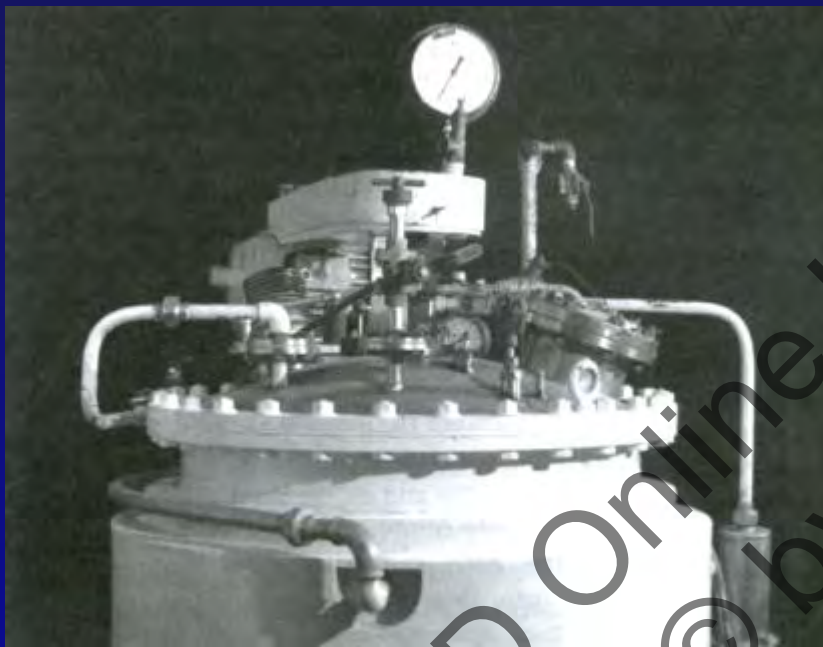
6-aminopenicillanic acid (6-APA)

1957 Discovery of penicillin nucleus



Semi-synthetic penicillins

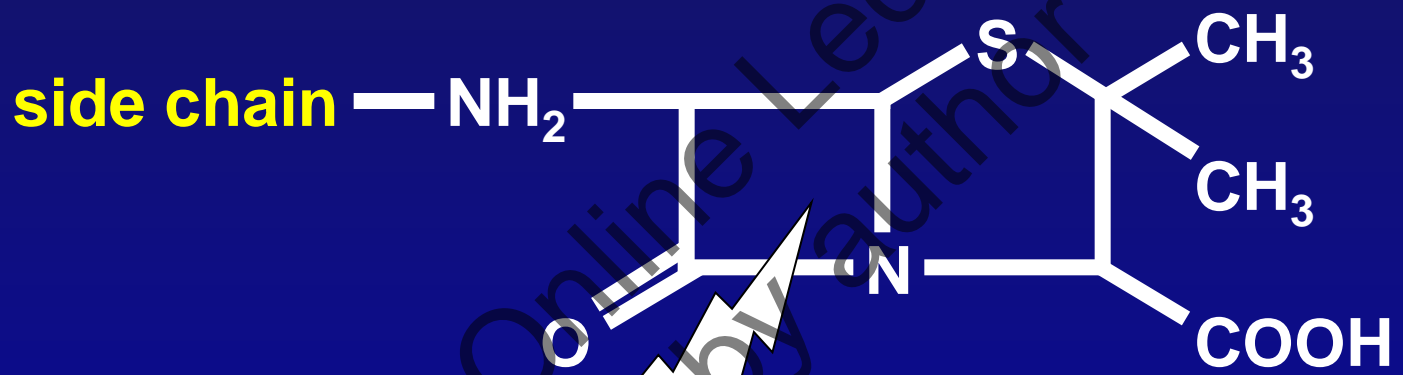
The Discovery of 6-APA : 1957



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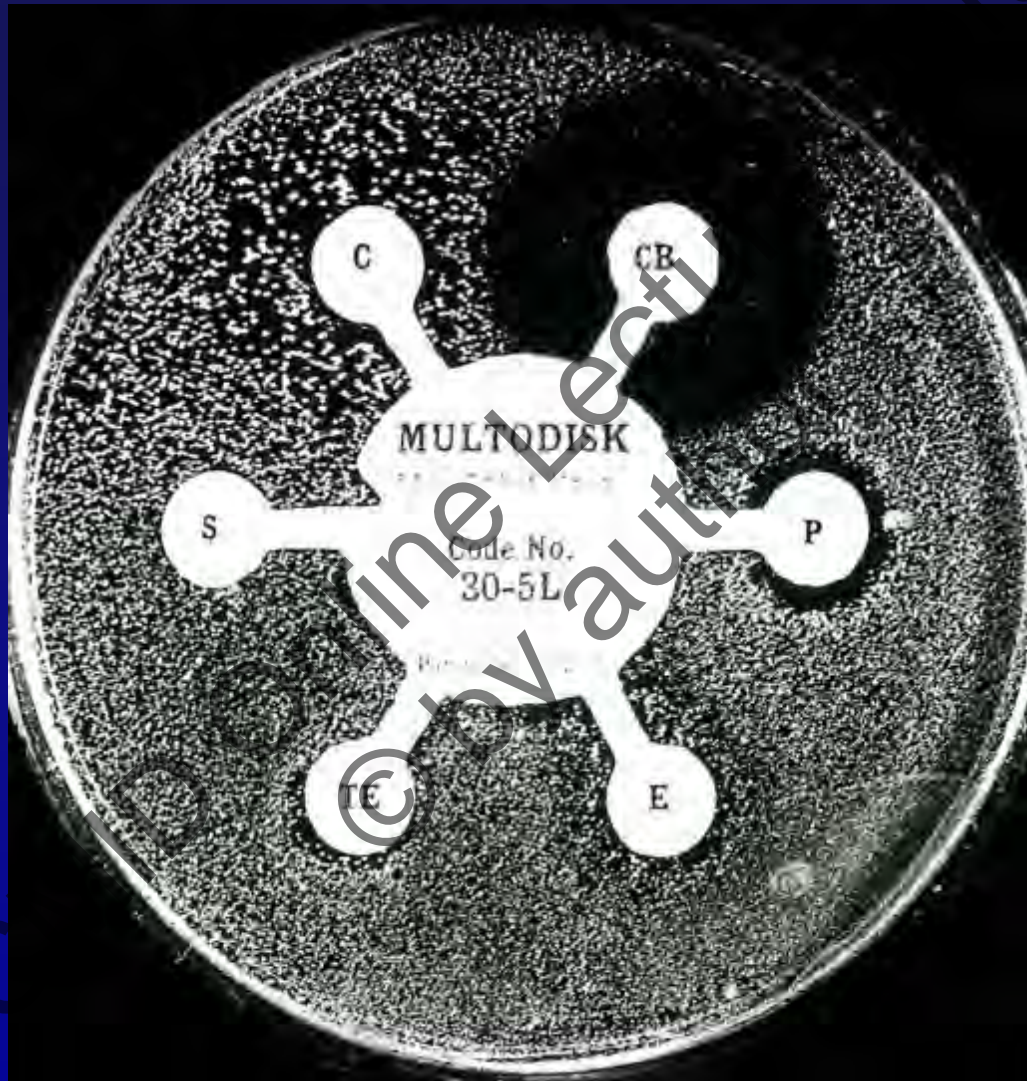
LP Garrod. *British Medical Journal*, 1956

“The most disquieting feature of present-day hospital practice is the prevalence of antibiotic-resistant staphylococcal infections.”



penicillinase
(β-lactamase)

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1960 – methicillin

“the first penicillin effective against resistant staphylococcal infections”



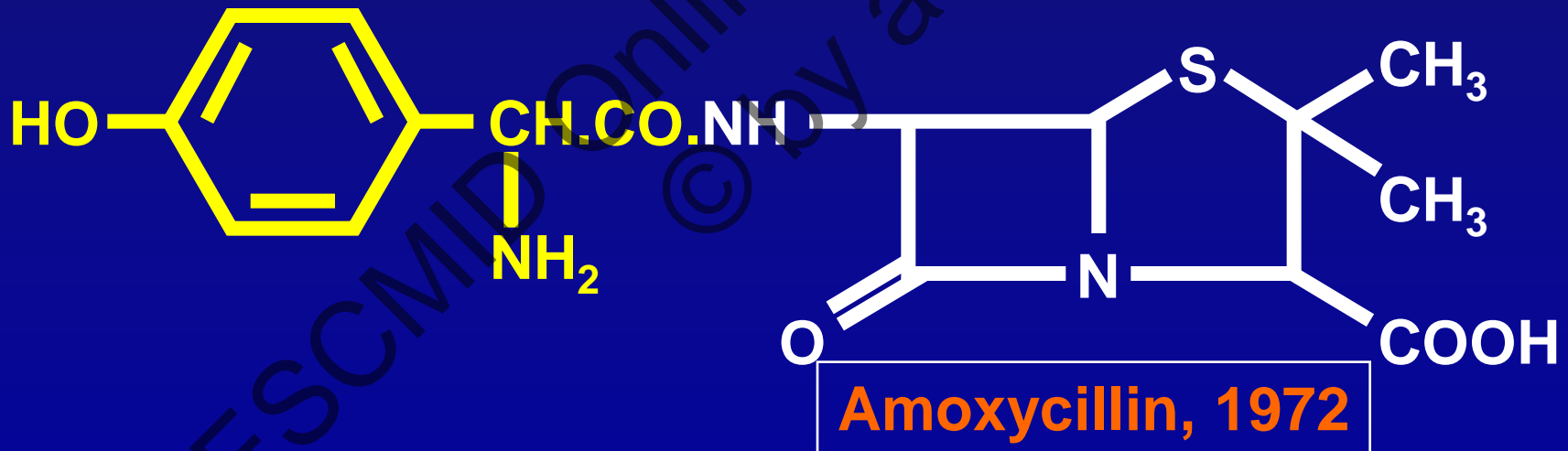
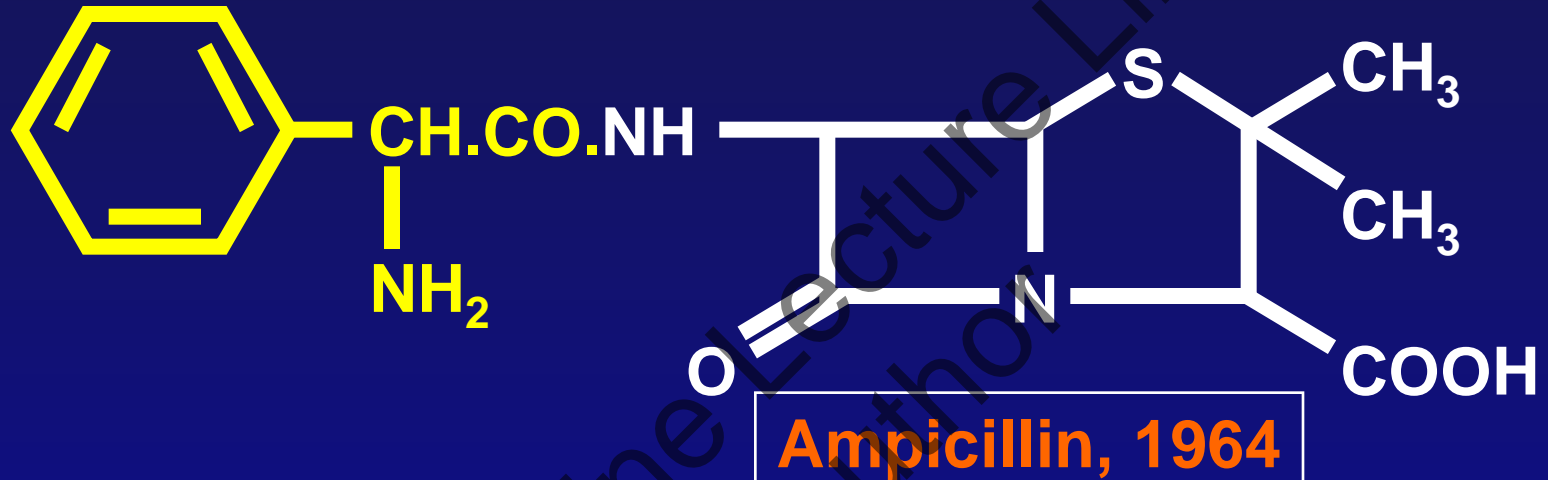
CELBENIN
first and still foremost
in the treatment of
serious staphylococcal infections

1960
*The first penicillin
effective against
resistant staphylococci*

1966
*Still the most proven
antibiotic against
serious staphylococcal infections*

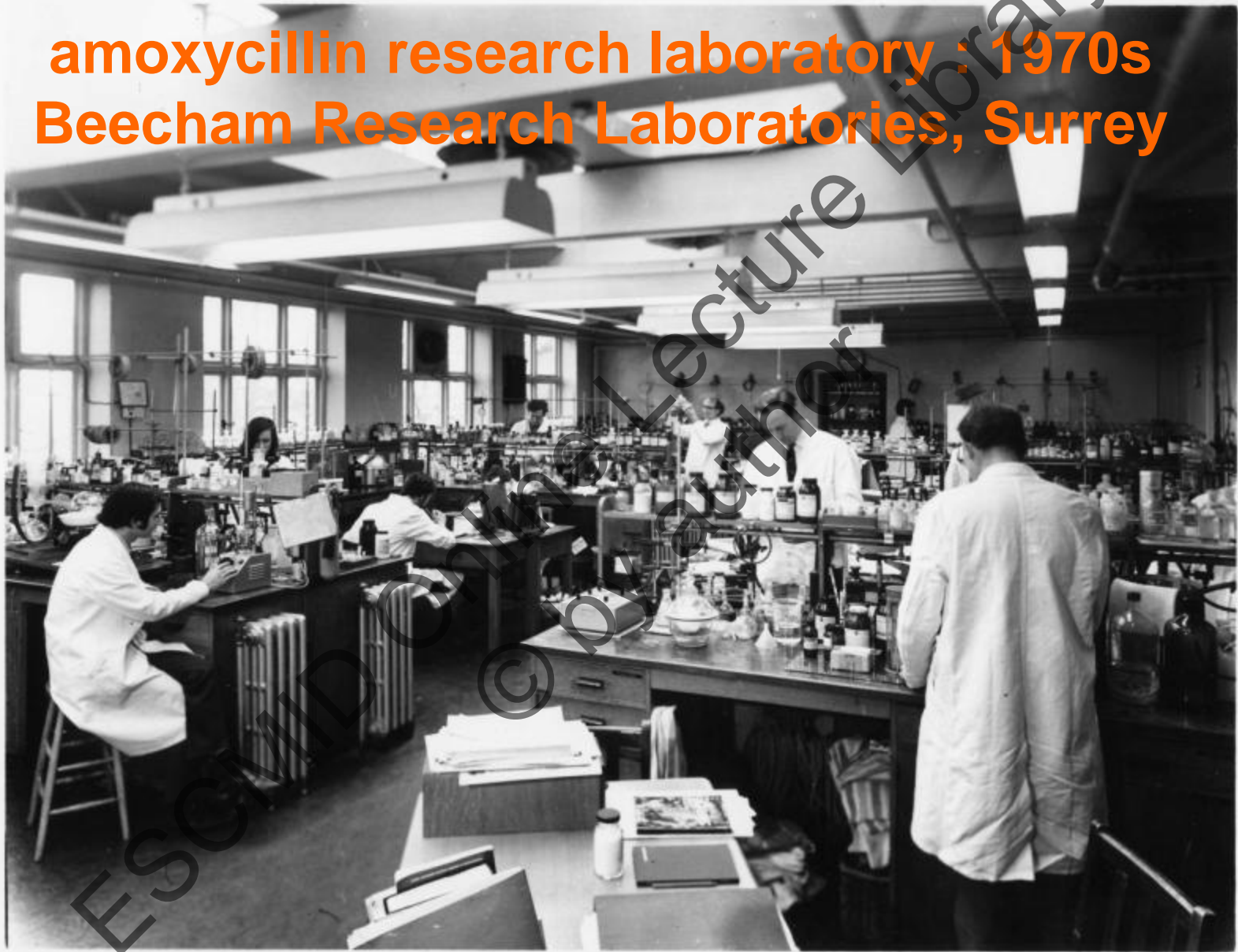
CELBENIN is a product of
Beecham Research Laboratories
Brentford, England 

New penicillins effective against other bacteria (E.coli, Salmonella, Haemophilus)

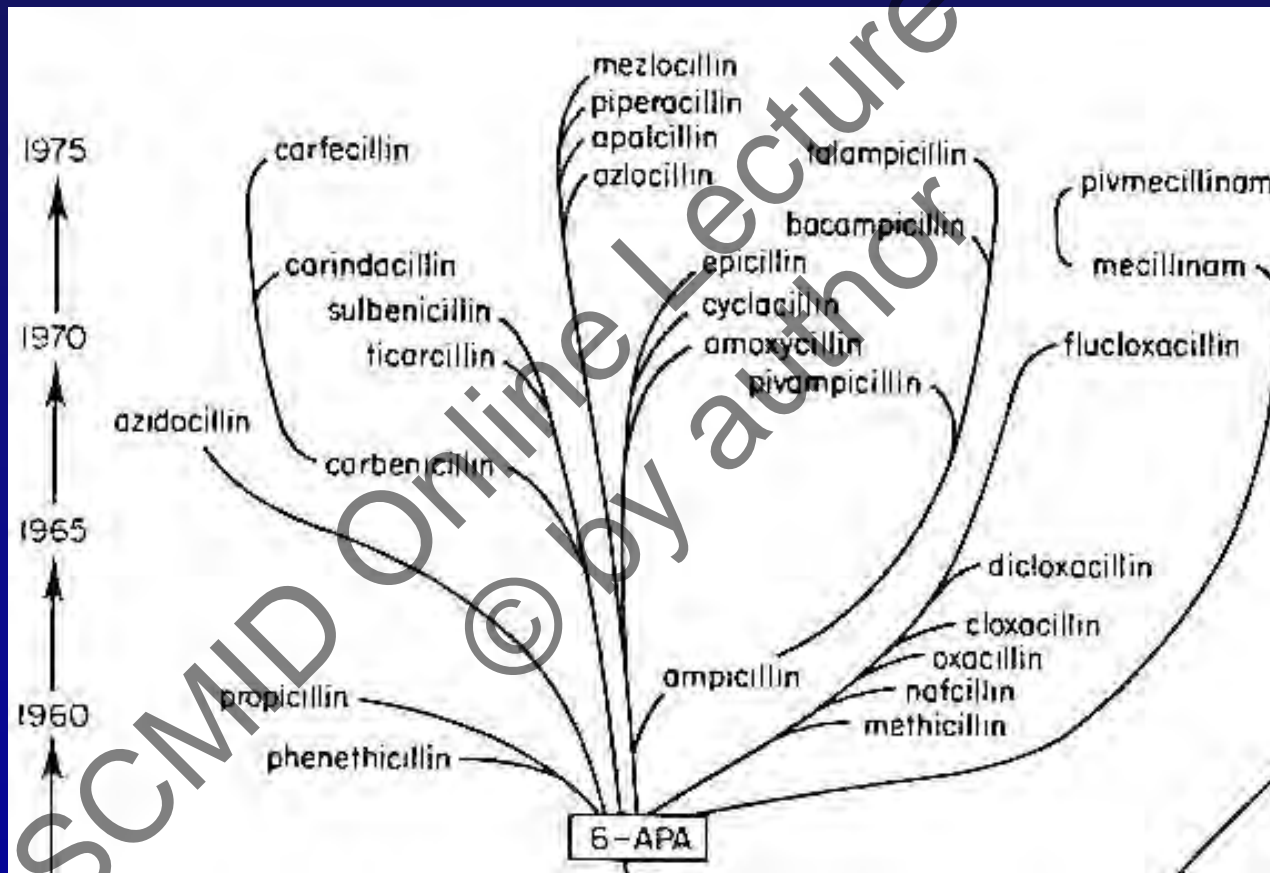


Blood levels twice as high from amoxicillin tablets

**amoxicillin research laboratory : 1970s
Beecham Research Laboratories, Surrey**



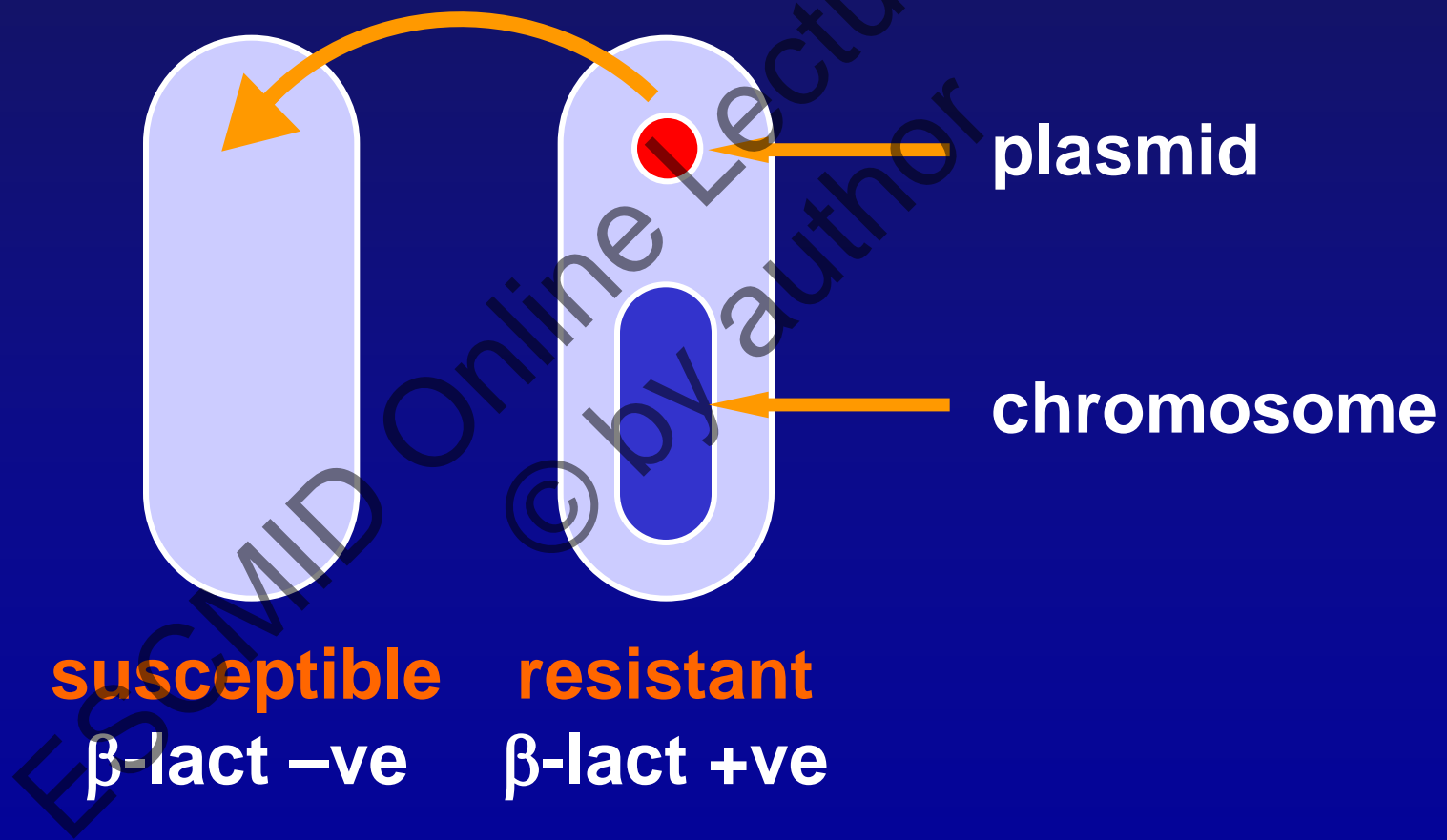
The penicillin 'tree'



Transfer of Resistance

	β -lactamase	
	negative	positive
<i>H. influenzae</i>	→ 1974	→
<i>N. gonorrhoea</i>	→ 1976	→
<i>M. catarrhalis</i>	→ 1977	→

1965 - Transfer of genetic code for resistance (β -lactamase production) from species to species.....

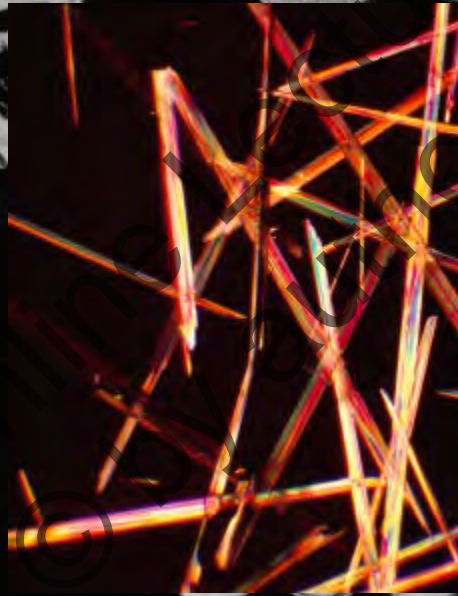


1967 – Screening microorganisms for β -lactamase inhibitors

1968 – MM4550, produced by *Streptomyces olivaceus*, culture 1627

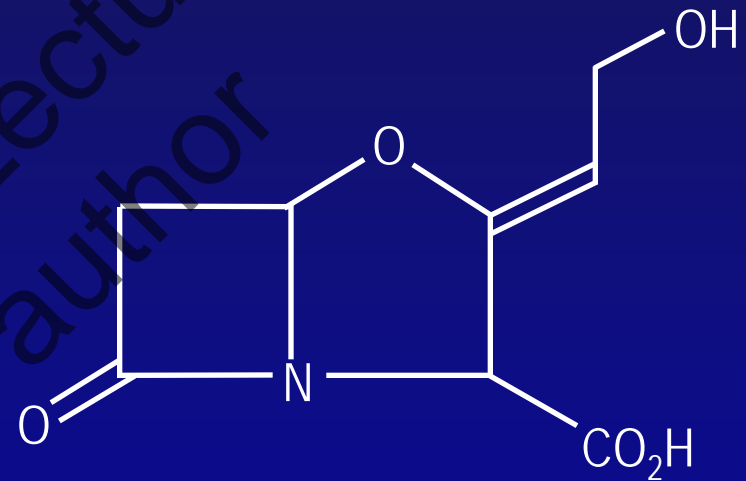
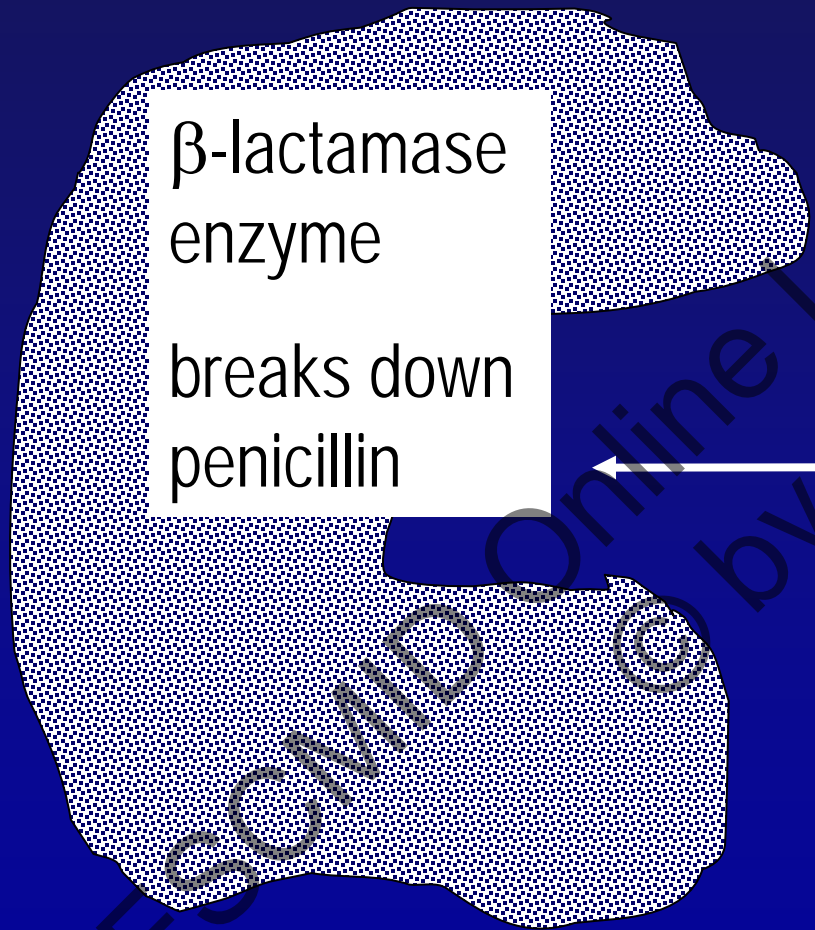
1972 – Clavulanic acid, produced by *Streptomyces clavuligerus*

**Compounds to block resistance
(β -lactamase inhibitors)
1972 : clavulanic acid**



from *Streptomyces clavuligerus*

Blocks site which would break down penicillin



Clavulanate

1981: amoxicillin + clavulanic acid (Augmentin)

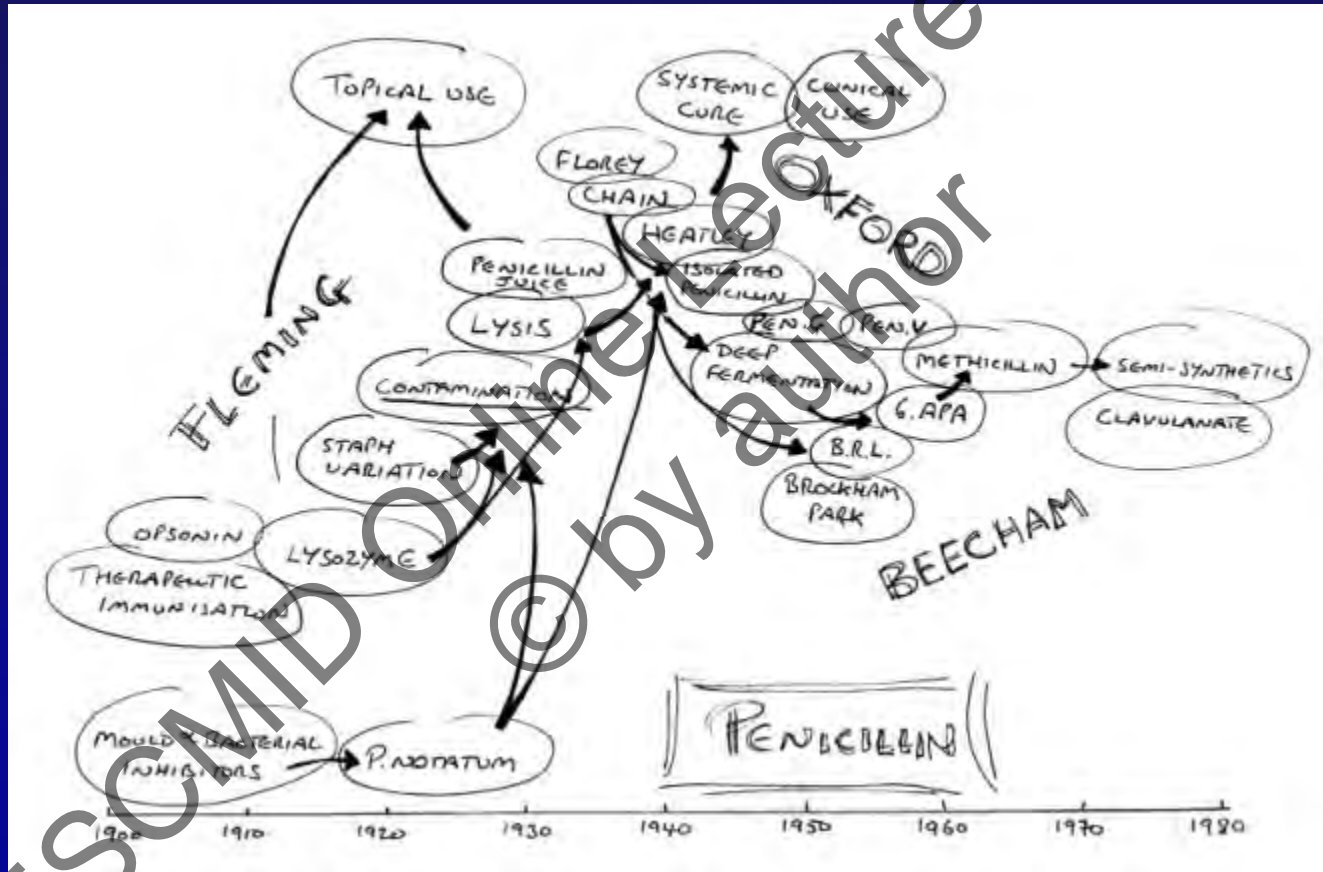


Retains activity vs
streptococci
pneumococci

Restores activity
against
staphylococci,
E.coli, H.influenzae
M.catarrhalis

Adds activity against
Klebsiella and
Bacteroides

Penicillin and serendipity?



Penicillin and Serendipity?

- No 'penicillin' without contaminating combination of Staph and *P.notatum*.....and right conditions
 - ◆ Flemings reports of lysis and penicillin
- No therapeutic penicillin without Oxford Group, lysozyme/lysis and natural occurring inhibitory substances
 - ◆ Isolation and proof of efficacy critical (....did they need Flemings findings?)
- No penicillin family without Chain/fermentation, BRL and 6-APA
- **No penicillin without prepared minds, vision, team work, hard endeavour and determination.....**
.....and some serendipity