# Early descriptions of antibiosis

WILLIAM FOSTER, M.R.C.G.P.
General practitioner

ALAIN RAOULT, Ph.D.
Bureau of Drugs, Ontario, Canada

The " discovery " of penicillin by Fleming in 1928 and its dramatic production, urged on by the necessities of war, heralded a new era of therapeutics. It has changed the pattern of disease the prognosis of infections, the expectation of life, indeed, it has changed the whole human ecology.

Before the discovery of penicillin, there were many early descriptions of the characteristics and properties of the *penicillia* and other moulds. Mycologically *penicillium* had been so described for 170 years, but in history and prehistory the characteristic green mould had been long associated with bad air, death, and putrefaction.

The name *Penicillium* was first given to a genus of fungi by Link in his *Observationes* in 1809. The genus was described briefly and three species enumerated *Penicillium glaucum, Penicillium candidum* and *Penicillium expansum.* It is not possible from nineteenth-century descriptions to identify the moulds *P. glaucum* or *P. candidum* except to recognise the familiar " penicillus " or brush characteristic of the penicillia. It is regrettable that Link and subsequent workers abandoned the use of the term *P. expansum* to differentiate some of the penicillia and called all the green penicillia moulds *Penicillium glaucum*! Thus the use of this nomenclature in early scientific papers gives no indication of the true identity of the *Penicillium* described.

The antibacterial activity of some green moulds belonging to the genus *Penicillium* had been known to investigators at least since the nineteenth century and this is also true of various spore-forming and non-spore-forming organisms. There were early and albeit crude attempts to use therapeutically the antibiotic effects of some micro-organisms, for many had perceived the possibility of using this natural function of conflicting microbes. Pasteur and Joubert (1877) inoculated bacteria into animals with anthrax; Cantani (1885) introduced *Bacterium termo* into the lungs of tuberculous patients whilst Gasperini reported the antagonistic effects of some of the actinomycetes. " Pyocyanase " an antibacterial agent was extracted from cultures of *Pseudomonas* by Emmerich and Law (1899) and a "streptothrix" with bacteriolytic action was prepared by the sterile filtration of an actinomycete by Gratia and Dath in 1924 and later by Welsh.

## Nineteenth-century research in England

The bacteriolytic properties of *Penicillium* were noted by Sturli (1908) and the lytic properties by Gratia in 1925 but the first scientific observations of the antagonistic actions of various micro-organisms were made long before, in England, in the preceding century by William Roberts of Manchester (1874) and John Tyndall of London (1876).

Roberts described his experiments and observed that bacteria would not grow easily in prepared media covered with *Penicillium glaucum.* Two years later Tyndall gave a detailed account of the antagonism between moulds and bacteria and the antibacterial properties of the penicillia moulds.

### *William Roberts*

William Roberts was born in 1830 in the village of Bodedern six miles east south-east of Holyhead on the island of Anglesey. He studied at University College, London and in Paris and Berlin. He became a physician to the Royal Infirmary at Manchester, successively a lecturer in anatomy and physiology, pathology, the practice and principles of medicine, and ultimately a professor of medicine at the Victoria University of Manchester.

### *John Tyndall*

John Tyndall is known to physicists for his brilliant research on heat, light, and sound, but few

*Journal of the Royal College of General Practitioners,* 1974, **24,** 889—894

are aware of his discoveries and research in the fields of biology and microbiology and of his major contribution to the problem of causation of infections. Tyndall was born in 1820 at Leighlin Bridge, County Carlow, Ireland and after a brief career as a surveyor obtained a doctorate from the University of Marburg in 1850. In 1853 he was appointed a professor of natural philosophy at the Royal Society, he was a colleague of Michael Faraday, became superintendent in 1867 and retired in 1887 after 34 years of scientific work in that institution. He investigated the transparency and opacity of gases and vapours, established the absorptive power of clear aqueous vapour; he conducted brilliant experiments explaining the blue of the sky and discovered the precipitation of organic vapours by means of light. In an amazingly productive scientific career, he made a most important contribution to the clarification of the aetiology of infection by calling attention to the fact that germ-free air did not initiate putrefaction, that infections did not arise by " spontaneous generation " but are caused by " the spread of germs."

Both Roberts and Tyndall indicated that the *Penicillium* moulds had some property or had an activity which inhibited bacterial growth and it could be claimed that they first " discovered ", " rediscovered " or " elucidated " the antibiotic activity of some moulds.

### Research in the seventeenth and eighteenth centuries

The celebrated Dutch draper, Antonie van Leeuwenhoek (1632–1723), is considered the founder of microbiology. He was the first, as far as we know, to observe micro-organisms, following his invention of the simple microscope. After his death it was found that his microscope was made of a " small double convex glass lens held into a socket between silver plates riveted together "; that the lenses were of carefully selected glass, and that the magnification was approximately 200 times. His scientific work is contained in 200 letters addressed to the Royal Society, the first being written in 1673. The original descriptions of micro-organisms are in the 18th and 39th letters, dated 9 October 1675 and 17 September 1683.

During the eighteenth century, the existence of protozoa was confirmed by Louis Joblot (1645–1723) and Otto Friederich Müller (1730–1784). Müller classified bacteria according to the nomenclature of Linnaeus (1707–1778). Antoine Laurent de Lavoisier (1743–1794) investigated the characteristics and functions of brewer's yeast. In his *Traité élementaire de Chimie* (1789), he showed that sugar on fermentation gave rise to equal proportions of alcohol and carbon dioxide. This work was further developed by Gay-Lussac (1815) and later by Baron Charles Cagniard-Latour (1836). As the biological nature of fermentation had been discovered, however, this explanation was unacceptable to many of the illustrious men who followed Berzelius, Wöhler, and Liebig.

### Pasteur

Analytical and critical scientists were engaged in this controversy for a considerable time and it only ended with the decisive influence of Louis Pasteur (1822–1895). The work of Pasteur had revived the old quarrel about " spontaneous generation " with its concept that infections arise " de novo ". This age-old debate dated from Aristotle and had the support of Buffon, Needham, Spallanzani, Lamarck, Müller and Kützing, to name but a few! It was part of the dogma of the intellectual establishment for centuries and its correction evoked discourse, disagreement, and discord. Pasteur devised careful, confirmatory experiments (1860–1864) which confounded the concepts of Pouchet, Joly, and Musset. In this endeavour he received the invaluable support of Tyndall. That there was a great interchange of ideas and information between the Royal Society and the Académie des Sciences de Paris is apparent from a perusal of the records of those learned societies. There existed a scientific *entente cordiale*, indeed had a number of common members and included, among the attending and corresponding members, most of the scientific minds of Europe.

At the meeting on 17 July 1876 of the Académie des Sciences, Pasteur brought to the notice of members the activities of the " great English physicist and his refuting of the theory of spontaneous generation "—

Communication du Dr Bastian, de Londres; par M. L. Pasteur.

" L'Académie a reçu dans sa dernière séance une Note du Dr Bastian, partisan déclaré de la génération spontanée, et dont les écrits ont eu, cette année même, l'honneur d'une réfutation, devant la Société Royale de Londres, par le célèbre physicien anglais Tyndall."

## Heat-sensitive and heat-resistant bacteria

In an age when the evils of specialisation were largely unknown, Tyndall did not confine himself to the fields of physics and chemistry but became deeply involved with the biological problem of spontaneous generation. He was also interested in the thermoresistance of germs and considered they could exist in a thermosensitive and a thermoresistant form. This hypothesis became firmly established in 1887 when, simultaneously but independently, Tyndall and Ferdinand Cohn (1828–1898) showed that hay-infusion (reasonably *Bacillus subtilis*) produces spores resistant to heat, *but* able again to germinate and to produce the heat-sensitive vegetative form. An application of this discovery is " tyndallisation " (Senez), the established fractional sterilisation method. Much of Tyndall's work on bacteria was originally published in the *Philosophical Transactions* but subsequently appeared in his *Essays on the Floating-Matter of the Air in Relation to Putrefaction and Infection* published in London in 1881 and in New York in 1882. The book was recently reprinted (1966) in a series devoted to the re-issue of the most significant literary landmarks in the evolution of scientific thought and achievement.

## First observations of antibiosis

William Roberts was also pre-occupied with the problems of microbial antagonism. In an astonishing paper, published in 1874, Roberts wrote:

> " the avoidance of air-contamination is important for another reason. The air is admitted, by most observers, to be highly charged with fungoid germs, and the growth of fungi has appeared to me to be antagonistic to that of bacteria, and vice versa. I have repeatedly observed that liquid in which the *Penicillium glaucum* was growing luxuriantly could with difficulty be artificially infected with bacteria; it seemed, in fact, as if this fungus played the part of the plants in an aquarium, and held in check the growth of bacteria, with their attendant putrefactive changes. On the other hand, the *Penicillium glaucum* seldom grows vigorously, if it grows at all, in liquids which are full of bacteria. It has further seemed to me that there was an antagonism between the growth of certain races of bacteria and certain other races of bacteria.
>
> On the panspermic theory it may be assumed that, what takes place when an organic liquid is exposed to the contamination of air or water, is this: a considerable variety of germinal particles are introduced into it, and it depends on a number of conditions (composition of the liquid, its reaction, precedence and abundance of the several germs) which of these shall grow and take a lead, and which shall partially or wholly lie dormant and unproductive. There is probably in such a case a struggle for existence and a *survival of the fittest.* And it would be hazardous to conclude because a particular organism was not found growing in a fertile infusion, that the germs of the organism were really absent from the contaminating media."

This explicit description must be regarded as the first recorded scientific observation on antibiosis, i.e., the antagonistic effect of a *Penicillium* on the bacteria. It preceded the discovery and development of penicillin and the antibiotics by at least 60 years.

Even more interesting observations were made by John Tyndall and his series of experiments are described in his essays. The first experiment consisted in setting up 40 tubes* containing infusions of partridge, pheasant, snipe, hare, sheep's heart, and codfish, five tubes being devoted to each, with four tubes of plover, three of mullet, and three of liver. The tubes were exposed to the air and it was noted that " the first two days produced no visible change in the pheasant infusion, while in two of the hare-tubes putrefaction had vigorously set in. Three days' exposure caused only one of the pheasant infusion to yield: four of the hare-infusion had yielded in the same time. The difference between them was also illustrated by the mould upon their surfaces. Some days after their exposure four of the five pheasant-tubes, were thickly covered with *Penicillium,* while the five hare-tubes, with one exception, which could hardly be considered such, had repelled that enemy, maintaining their bacteria undisturbed."

This clearly was Tyndall's initial observation of the antagonism which existed in his preparations of bacteria and *Penicillium.* However, the results were not quite clear in the sense that the pheasant-tubes behaved differently from the hare-tubes. A possible explanation of this apparent discrepancy is given later.

Further in the book, Tyndall studied the influence of temperature on putrefaction and noticed that one of the mutton-tubes set up in his study " gathered over it a thick blanket of *Penicillium.* On 13 December it had assumed a light brown colour, " as if by a faint admixture

---

*The tubes used were glass flat dishes and were introduced later by Petri (1852–1921).

of clay "; but the infusion became transparent. The ' clay ' here was the slime of dormant or dead bacteria, the cause of their quiescence being the blanket of *Penicillium* ". " I found no active life in this tube, while all the others swarmed with bacteria " concluded Tyndall.

The crucial experiment was carried out on 23 October 1875 when a tray of 100 tubes was exposed to air. The tray comprised 30 tubes of hay-infusion, 35 tubes of turnip-infusion, and 35 tubes of beef-infusion.

Illustrative plates accompany the description of the experiment and show the development of bacteria in the various tubes. The tray of tubes was examined on 26 October at noon and 1900 hours, on 27 October at 1830, and on 29 October at 1030. A final examination was done on 31 October where it was found that only four out of the 30 hay-infusion tubes were free from mould: " The *Penicillium* was exquisitely beautiful " exclaimed Tyndall who found three types of *Penicillium* " struggling for existence ". Nine of the turnip-infusion tubes were free from mould as were 17 of the beef-tubes. " The mould-developing power is obviously greater in the hay—less in the turnip—and least of all in the beef-infusion " Tyndall continues and finally remarks that " in every case where the mould was thick and coherent the bacteria died, or became dormant, and fell to the bottom as a sediment. The growth of mould and its effect on the bacteria are very capricious ".

In yet another experiment done in the middle of November of the same year, Tyndall noted that an " absence of uniformity was manifested in the struggle for existence between the bacteria and the *Penicillium.* In some tubes the former were triumphant; in other tubes of the same infusion the latter was triumphant! "

It is clear from these experiments that Tyndall, after Roberts, and perhaps in a more comprehensive fashion, observed the antagonism that prevails between bacteria and *Penicillium.* Of course, one wonders with which strains of bacteria and penicillia Tyndall worked. It is impossible to establish, categorically, the species described, but one can assume that he was handling several strains or even species of each. This may explain the apparent inconsistency of the results, if the quantities of penicillin-sensitive bacteria varied as well as the quantities of penicillin-producing penicillia. The amount of each would therefore determine the fate or outcome of each tube, in a standard set of conditions and without sensitivity-variance. It seems certain that both Roberts and Tyndall worked with penicillin-sensitive bacteria. It is more difficult to ascertain whether or not they handled specifically *Penicillium notatum* or *Penicillium chrysogenum*, but this need not necessarily be the case, as many other strains of penicillia produce penicillin-like substances (Raper and Thom, 1949) usually in lesser quantities.

Nevertheless credit and recognition is due to both Roberts and Tyndall for their independently recorded observations of 1874 and 1875 of phenomena hitherto unrecognised by scientists. It is unfortunate that the rest of the scientific world and the subsequent generations failed to take notice of this demonstration of the naturally-occurring antagonists to bacteria. In the expansion and multiplication of bacteriological and scientific knowledge which followed, no importance was placed on their discovery and it passed into oblivion. It was not re-examined until Fleming, dealing with a particularly potent strain of *Penicillium notatum* and working with totally different and improved equipment and techniques, was able to demonstrate again this activity and to name the antibiotic substance penicillin.

## Penicillin

In 1928 at St Mary's Hospital London, Fleming observed that a culture plate of staphylococcus was unexpectedly contaminated by *Penicillium notatum.* The colonies of the staphylococcus in the neighbourhood of the mould were destroyed and appeared to be undergoing dissolution. The mould was isolated; it was found to produce a substance with an antibiotic effect and subsequently in 1929, Fleming named the substance penicillin.

Ten years later Florey and his Oxford co-workers were to investigate antibiotics and selected penicillin. The actual emergence of penicillin as a therapeutic agent began with the publication in 1940 of the paper by Florey *et al.* in which they described the curative properties of this amazing antibiotic and their development of adequate separation techniques.

The next problem to be solved was that of large scale production and with the escalation of war-time needs and the importance given to the project the progress was dramatic. Since that period newer penicillins have been developed and newer antibiotics have been discovered, but nothing can compare with the brilliance and application of the 1945 Nobel Laureates, Fleming, Florey, and Chain and their host of co-workers in the successful production of the first of the antibiotics.

Tyndall and Roberts, years before, had indicated and demonstrated the antibacterial action of the *Penicillium* moulds. However, as in so many fields of human endeavour, valuable theories are often expounded, precious facts elucidated and truths demonstrated, the significance of which is not readily apparent and the knowledge of them lost, only to be rediscovered years later.

Tyndall and Roberts corresponded regularly with each other and Tyndall gave due recognition to Roberts' original observations. Their correspondents included members of the Royal Society*, fellow-scientists and Professor Thomas Huxley. Huxley, writing to Tyndall in 1875 commented that there is " Nothing new under the sun " and he added a quotation about the subject under discussion from Ehrenberg's " Infusionsthierchen " 1838 p. 525.

" uebrigens kann man sich die in der Atmosphäre Schwimmenden Thierchen wie Wolken denken, mit denen ganz leere Luftmassen, ja ganze Tage Völlig reinen Luftverhältnisse wechseln."

Tyndall was surprised at the similarity of phraseology used by this German scientist 40 years before, for he knew nothing of Ehrenberg's concepts and was astonished to find that he had written of " little animals swimming in the atmosphere as in changing clouds!" Thus the theory of airborne infection was not new even in the mid-nineteenth century, although it was to be proven by Pasteur, Tyndall, and others.

## Ehrlich

There are in the folklores of the world, many stories about the healing powers, the magical powers of moulds and fungi, and of specific treatments and specific cures of a semi-mystical nature. It was Ehrlich, however, who clearly, rationally, and scientifically enunciated the concept of the " magic bullet ", the " specific chemotherapy of disease " (Lechevalier and Solotoroucky, 1965). Chemotherapy began with the exploitation of natural products and Ehrlich was ready to point out that " these very substances with the greatest powers of healing were originally discovered by the detective instincts of primitive peoples ". Thus like so many investigators he recognised that valuable therapeutic agents were to be found in some of the " home-remedies " of folk-medicine and that specific cures existed before scientific explanations of their mode of action were possible. The second stage of chemotherapy began with the synthesis in laboratories of chemical substances specific to certain diseases and the story of Ehrlich's successful search for Salvarsan is now well known. Erlich also anticipated the third phase of chemotherapy; that is the use of micro-organisms to synthesise naturally occurring substances in amounts greater than produced normally; in amounts large enough to be used therapeutically.

In 1899 the Institution for the Investigation and Control of Sera was transferred to Frankfurt and a second institute, the George Speyer House dedicated entirely to chemotherapy, was built next to it. At the dedication, Ehrlich clearly defined chemotherapy and chemotherapeutic substances which would "exert their full action exclusively on the parasite harboured within the organism . . ." Thus he focused attention on the need for the direction of pharmacological research, being unaware of the many observations already made in this primitive field of the study of the antibiotic activities of some organisms. Thus the era of chemotherapeutics began with the twentieth century ignorant of early discoveries. There is now a plethora of antibiotics with all their blessings, their many unwanted hazards, and the concomitant iatrogenic disease.

The efforts, experiments, and investigations made by so many in the previous century all added to the sum of pharmacological knowledge and thus all helped to produce the changes and advances which ultimately resulted in the development of the antibiotics.

*Charles Darwin, Sir John Lubbock, Mr Siemens, Mr Rollo Russell and Dr Joseph Dalton Hooker, friends and contemporary members of the Royal Society, collaborated with Tyndall by taking prepared tubes of infusions to agreed locations outside London and returning them " cloudy and peopled with bacteria."

That some of the knowledge rediscovered had been postulated years before should but remind us of the gay milliner to Marie Antoinette, Mademoiselle Bertin (circa 1785) and her dictum " There is nothing new under the sun except what is forgotten."

*Nil dictum quod non dictum prius.*

### REFERENCES

Chain, E., Florey, H. W. Yardner, A. D., Heatley, N. G., Jennings, M. A. Orr-Ewing, J. & Sanders, A. F. (1940). *Lancet*, *2*, 226–228.

Compte-Rendus (1862–1881). *Academie des Sciences*, Paris.

Compte-Rendus (1862–1881). *Philosophical Transactions of the Royal Society*, London.

Compte-Rendus (1876). *Academie des Sciences*, Paris, **83**, 176.

Darwin, C. (1859). *Origin of Species*. London: J. Murray.

Fleming, A. (1929). *British Journal of Experimental Pathology*, **10**, 226.

Lechevalier, H. A. & Solotorovsky, M. (1965). *Three centuries of microbiology*. New York: McGraw-Hill.

Raper, K. B. & Thom, C. (1949). *A manual of the penicillia*. p. 102. Baltimore: Williams & Wilkins.

Roberts, W. (1874). *Philosophical Transactions of the Royal Society*, London, **164**, 457, 466.

Senez, J. C. (1968). *Microbiologic generale*. Paris: Deren et cie, Editions Doin.

Tyndall, J. (1876). *Philosophical Transactions of the Royal Society, London*, **166**, 27.

Tyndall, J. (1881). *Essays on the floating-matter of the air in relation to putrefaction and infection*. London: Longmans i (1882). New York: D. Appleton and Co. i (1966). New York and London: Johnson Reprint Corp.

---

## ACCIDENTS TO CHILDREN

A recent report by the National Children's Bureau shows that a quarter of all children have had a serious accident by the age of 11.

The commonest place for accidents is in the home, with burning and scalding the most frequent injuries.

The most vulnerable children are those in large or poor families.

---

## VOCATIONAL TRAINING FOR GENERAL PRACTICE

The views of 89 vocational trainees were compared with those of 45 doctors concerned in schemes of vocational training for general practice. Both groups agreed on most points, except on the desirability of compulsory vocational training: 42 of the trainees thought this to be desirable compared with 35 of the teachers.

The composite scheme favoured by both trainees and teachers offered an initial period in general practice with an organised course of seminars throughout the training period. The subjects in which most considered hospital experience essential were paediatrics, general medicine, and obstetrics and gynaecology.

### REFERENCE

Drinkwater, C. K. (1972). *British Medical Journal*, **4**, 96. (Author's summary).