Quarterly Newsletter of the Belgian Society for Microbiology
Issue no. 11, September 2015

Contents

Welcome

Dear Microbiologist,
I am pleased to send you the 11th issue of the BSM Newsletter. It contains several interesting items for which I thank the contributors. I also want to stress your attention to BSM's annual meeting (11th December 2015) "Microbes and the Global change", a topic of major scientific as well as social importance. During the last decade a huge debate has started with respect to the possible future impact of climate change and the increased mobility on health and economy. This "global change" can trigger the spread of microbes, as recently already illustrated (SARS, Ebola, ….). Among others, vectors that cause diseases like dengue and leishmaniasis are now moving to originally less warm regions. This may lead to the introduction of tropical diseases in temperate climate regions. Microbes also play a pivotal role in the natural cycle of carbon and nitrogen. It is therefore important to understand the biological mechanisms that regulate these elements and their interactions on land, in oceans and in the atmosphere. This can give us more insight on the impact that climate and other global changes can have on the metabolic activity of the microbes and thus the cycle of these elements. During the annual symposium several aspects of "global change", microbial threats and opportunities will be highlighted by renowned scientist. Besides invited lectures, a poster session (all microbiological topics are welcomed) will be held. Of the submitted poster abstracts (deadline **25 Nov 2015**), 6 of them will be selected for oral presentation. Moreover, at the end of the meeting best posters will be awarded. More details on the program can be found in this issue. How to register to the symposium, is explained on the following page and on the BSM website (www.belsocmicrobio.be).

I also would like to draw your attention to the opportunity BSM members have to apply for FEMS grants to visit another laboratory in Europe, or to attend or organize a meeting, since FEMS supports microbiology with Grants as well as by publishing articles free of charge in the 5 FEMS Journals. FEMS aims to advance and unify the field of microbiology by supporting microbiology research, education, training and continuing professional development, translating microbiology research and knowledge into policy, making economic and social impact, internationalising European microbiology.

If not yet done, renew or join BSM membership and register to the symposium!

Jozef Anné, BSM President

News from FEMS

FEMS is the Federation of European Microbiological Societies, and its main mission is to advance and unify microbiology knowledge. FEMS brings together 53 member societies from 36 European countries, covering over 30000 microbiologists. Belgium is represented in FEMS by BSM, and our FEMS delegate is Paul Cos (UAntwerpen).

At the 6th Congress of European Microbiologists (FEMS 2015) 7-11 June 2015 in Maastricht, one of the workshops was **Scientific Publications explained.** The presentations of this workshop are available for download here.

The **7th Congress of European Microbiologists** is one of the leading meetings of its kind and will connect thousands of microbiologists from around the world. **FEMS 2017** will feature symposia and workshops led by prominent scientists in their respective fields. The goal of this international gathering is to provide a comprehensive forum for the exploration and discussion of various topics in microbiology. **FEMS 2017 will be held 9-13 July 2017 in Valencia, Spain.**

Call for FEMS grants applications

Members of FEMS Member Societies can apply for research fellowships and/or support when organizing a meeting.

FEMS Research Grants. Applicants should be active microbiologists, having obtained their highest degree less than five years prior to the application deadline date or be a PhD student. They should be a member of a FEMS Member Society, at least one year before applying and be a resident in a European country or a country that has a FEMS member. Other grants are: **FEMS Meeting Attendance Grants; FEMS Meeting Grants; FEMS National & Regional Congresses Grants.** For more information how to apply for these grants, go to the FEMS website (http://www.fems-microbiology.org).

Symposium organized by the Belgian Society for Microbiology and the National Committee for Microbiology

Microbes and the Global Change

Brussels, Academy Palace, 11th December 2015

08.30 *Registration – Poster mounting*

09.00 *Welcome address*

09.10 Alain Préat (Brussels, BE) - *Introduction to climate change*

09.45 Jan Semenza (Stockholm, SWE) - *Vulnerabilities to the risks of changes in infectious disease transmission caused by climate change: a modelling study*

10.30 Short communications selected abstract (2)

10.55 *Coffee break and poster viewing*

11.30 Eric Chatelain (SUI)- *Neglected diseases in a changing world*

12.10 Short communication of selected poster abstract

12.30 *Lunch and poster viewing*

(continued on next page)

Symposium organized by the Belgian Society for Microbiology and the National Committee for Microbiology

Microbes and the Global Change

Brussels, Academy Palace, 11th December 2015

(continued from previous page)

14.30 Albert D.M.E. Osterhaus Rotterdam, NL) - *Drivers of emergence and sources of future emerging and reemerging viral Infections*

Short communications of selected abstracts (3)

15.55 Mike Jetten (Nijmegen, NL) - *The role of microorganisms in relation to greenhouse gases.*

16.30 *General conclusions and presentation of best poster awards*

Meeting sponsors

Membership & registration for the annual symposium

Members paying **before 15/07** owe only € 25 and will get free access to the annual symposium and other events organized by BSM. They can also register at reduced rates for certain events co-sponsored by BSM.

Later payments for symposium pre-registration or for membership will be €30.

On-site registration fee will be €35.

To renew your membership and to register for the symposium visit the BSM website
www.belsocmicrobio.be

Belgian Society for Microbiology

100 Years ago: Discovery of the Bacteriophage(s) PART II: Richard Bruynoghe versus Jules Bordet in the bacteriophage debate

d'Herelle's first report on the bacteriophage phenomenon was presented in September 1917 to the *Académie des Sciences de Paris* by Emile Roux, then Director of the Institut Pasteur in Paris. Jules Bordet (1870 – 1961), as the Director of the Brussels Pasteur Institute, must have been among the very first to be informed about the work and the ideas of d'Herelle. Lysis of bacteria was a subject close to his heart as he had discovered how antibodies, after recognizing a bacterium, recruit complement to lyse it. No wonder that he did not like the idea of d'Herelle that the bacteriophage was a critical factor in defense against intestinal bacterial infections. In fact, he was more attracted to the idea of Tamezo Kabeshima that the bacteriophage was a humoral factor, e.g. some sort of lytic enzyme, produced by the bacteria themselves. Kabeshima had come to that conclusion because he had found that d'Herelle's bacteriophage failed to lose self-reproducing activity after heating at 70° C or after exposure to various antiseptics. Therefore, he proposed that the bacteriophage lacked all viability.

At the time, a Romanian scientist, Mihai Ciucă (1883-1969), was working in Bordet's laboratory. He was investigating the physiology and actions of leukocytes, which he regularly obtained from inflammatory exudates induced by injecting live *E. coli* in the peritoneum of guinea pigs. Emulating the example of d'Herelle he also tested whether the bacteria retrieved from the exudate would display the bacteriophagy phenomenon: with success. Thus, Bordet confirmed d'Herelle's observations using a different bacterium. He reported about these experiments in 1920 during the autumn session of the Belgian branch of the French *Société de Biologie* [1]. However, rather than accepting d'Herelle's claim that the lytic principle was an invisible microorganism, he opted for Kabeshima's idea of a secreted humoral factor.

Over the subsequent months he developed a comprehensive theory holding that bacteriophagy was an exaggeration of a normal autolytic process triggered in some of the bacteria by an exogenous stimulus (e.g. contact with leukocytes) and consisting of hypersecretion of a lytic enzyme that at the same time acted as an endogenous stimulus for other bacteria to do the same.

Over time Bordet's theory underwent slight modifications. In the very beginning he postulated that the humoral factor released by the primarily affected bacteria 'imprinted' fellow bacteria with a novel 'hereditary' character consisting of undergoing spontaneous autolysis. However, he soon abjured this quasi Lamarckian idea of a hereditary change. In the beginning he also considered the hypothetical factor as a lytic enzyme responsible for bacteriolysis; later he suggested it might as well be some sort of toxin that triggered autolysis (*'déclenchateur d'autolyse'*). Central to his theory was that the factor(s) responsible for (auto)lysis were not introduced from the outside but pre-existed in smaller quantities in unaffected bacteria. Bacteriophagy in his mind was nothing more than a runaway of normally occurring 'physiological' autolysis. Often he compared it to another purely humoral system that he had studied: the conversion of prothrombin to thrombin under the influence of thrombin itself: a simple positive feed-back loop.

In 1919 Bordet was rewarded with the Nobel Prize for Physiology and Medicine for his work on complement. This gave him a strong voice in the bacteriophage debate and, together with several other factors, made that the large majority of bacteriologists, including textbook writers, accepted his explanation. One notable exception was Richard Bruynoghe (1881 - 1957), Professor of bacteriology and hygiene at the '*Université Catholique de Louvain*'.

(Note: a short biography is available from http://www.md.ucl.ac.be/histoire/liste.htm).
Bruynoghe resolutely took the defense of d'Herelle's vision [2].

Bordet and Bruynoghe were different personalities in various ways. However, most important was their different training and scientific antecedents. Bruynoghe, 10 years younger, did not have the advantage of previous scientific achievements that Bordet could rely upon: discovery of the agent of pertussis (*Bordetella pertussis*); research on phagocytosis of bacteria by leukocytes, discovery of the complement system, studies on the mechanism of blood clotting. Bruynoghe's scientific experience was both shorter and narrower. After serving some time in a pathology lab, he had followed training in bacteriology in France and Germany and had then assumed the task to organize the practice and teaching of medical bacteriology at his university. Lacking research experience in diverse domains of physiology, he had the converse advantage not to be hindered by the biases imposed by these experiences. In a way, Bordet's scientific world had become one of theories, Bruynoghe's was to become one of down-to-earth practical reality: routine lab work and teaching.

Nevertheless, as a director of the 'Institut de Bactériologie' of his university he also assumed the task to create an environment for graduating M.D.'s to familiarize with laboratory practice and, for the more brilliant ones, scientific research in bacteriology and infectious disease. Over the years several future professors of medicine were to receive basic laboratory and research training under Bruynoghe's guidance. In the 1920s his young collaborators were Joseph Maisin (1893-1971), future professor of oncology and radiotherapy, René Appelmans (1896 - 1953), future professor of surgery and Paul Brutsaert (1898 – 1960), future director of the bacteriology lab at the Tropical Institute in Antwerp. They were the ones who did the experiments with which Bruynoghe challenged Bordet's enzyme theory.

Bruynoghe regularly attended and presented his work at the sessions of *Société de Biologie*. Within months after Bordet had given his first presentation on the bacteriophage, Bruynoghe reported to also have obtained a bacteriophage following the recipe of Bordet and Ciuca [3,4]. Over time between 1921 and 1923, he described a series of experimental results that either argued against the enzyme hypothesis, or at least were more easily explained by the microorganism hypothesis of d'Herelle. In fact, never was there any disagreement on the experimental data; divergence concerned only the interpretation. Furthermore, Bruynoghe never examined nor discussed the idea of d'Herelle that the bacteriophage played a role as a mechanism favoring spontaneous remission of infections such as bacterial dysentery. On that point he probably felt d'Herelle was completely wrong.

Briefly, Bruynoghe's arguments were as follows:

1. Adaptability of the phage and variability of virulence

Joseph Maisin showed that his phage primarily isolated from E. coli readily reproduced in *Shigella* cultures and became more prolific with further passage [5]. Later on, Paul Brutsaert also found that the phage could increase or decrease in virulence within its original host depending on the ratio between inoculums and substrate [6]. Such easy and rapid adaptability was a property that bacteriologists of the time were used to see with many microorganisms. Therefore, Bruynoghe felt that this was an argument to assume that the phage was a similar rapidly changing biological entity rather than an enzyme.

2. Conservation of antigenic specificity after adaptation.

Bruynoghe's collaborators immunized rabbits with lysates from phage-infected cultures and thus produced antisera that they found to be highly specific: antisera against phages originally isolated from an *E. coli* or a *Shigella* would not cross-neutralize their respective phages. With such antisera it could be shown that each phage conserved its antigenic specificity after passage in whatever other host bacterium [7,8].

This, in Bruynoghe's mind, could not be explained with Bordet's enzyme theory. In fact, at no time did Bordet refute this argument; he only vaguely replied by stating that the immense adaptability of bacteria could provide an explanation.

3. Behaviour of the minimal viable entity as an organized particle rather than a quantity of dissolved enzyme.

d'Herelle had shown that, at limit dilution of the phage suspension, co-inoculation with host bacteria on agar slants resulted in formation of plaques, the numbers of which were reciprocal with the dilution. This, in his mind was proof for the individual phages to consist of an organized particle. André Gratia, a close associate of Bordet, elaborately argued that this result could equally well be explained by the dilution of sensitive bacteria. René Appelmans, in Bruynoghe's lab, circumvented this objection by simplifying the experiment: instead of inoculating the 10-fold phage dilutions on agar slants, he inoculated them in broth. As expected, at the limit dilution, about 50% of the broth cultures showed lysis. However, in repeat experiments, every now and then one culture of a higher dilution showed lysis [9]. In Bruynoghe's mind this was only possible if the phage was an organized particle, not when it was a dissolved chemical.

4. Unlimited plurality of bacteriophages in Nature

Over time Bruynoghe's collaborators isolated numerous bacteriophages from different sources: bacteria isolated from sewage, from the intestines of horses, cows, pigs and chickens [10]. Most of them differed in thermosensitivity, host range and antigenic constitution. Bruynoghe felt that this unlimited plurality could hardly be accommodated in Bordet's enzyme hypothesis [2]. In a reply to this objection, Bordet stated:

'D'après M. Bruynoghe, il existerait dans la nature une foule de bactériophages. … En réalité, … les microbes sensibles … sont en voie de perpétuelle mutation. [Ce qui permet] d'expliquér les constatations singulières en rapport avec la pluralité des bactériophages, sans qu'il soit nécessaire d'invoquer le virus invisible de d'Herelle.'

Thus, Bordet seemed to propose that the number of bacteriophage enzymes possessed by bacteria is limited. The sensitivity of a bacterial species to bacteriophagy would be determined and limited by its endogenous repertory of phage enzymes.

5. Development of phage-resistant bacteria.

Bacterial cultures can survive exposure to phage such that they can even be subcultured. In that case they take a peculiar aspect: agar surface cultures form layers with a glassy aspect. From these layers the early bacteriophage workers could isolate bacteria from which subcultures could be grown, some of which had a normal aspect but nevertheless produced phage that killed the indicator strain and others that contained no detectable phage and were also resistant to re-infection with the original phage.

Bordet and Bruynoghe explained the 'emergence' of these different resistant variants (producers and non-producers) each within the framework of their own theory. For Bordet's enzyme theory the conversion of a lysis-sensitive to a lysis-resistant bacterium implied a change in the physiologic functioning of the endogenous enzyme system. He proposed that the incoming phage enzyme induced 'adaptive' changes, resulting in heterogeneity of the bacteria in terms of sensitivity to autolysis and capacity to produce the enzyme [11]. Interestingly, he considered the completely resistant ones as '… having been cured and become invulnerable' *('il s'agit de germes guéris et devenus invulnérables')*, a proposition which can easily be seen as genetic change directed by an external factor, the core element of Lamarckian evolution.

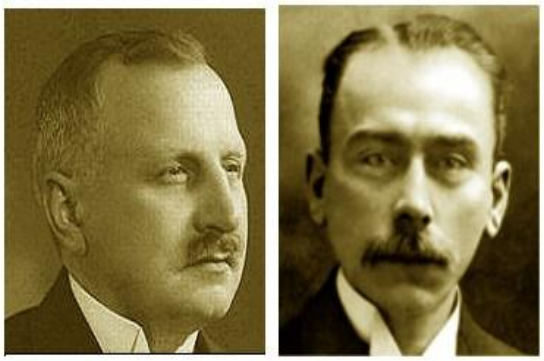
Bruynoghe, who saw the whole system as symbiosis between two microorganisms stated [12]: *'En admettant la théorie du virus ... on pourrait préciser ces deux degrés de résistance en considérant les premiers comme des immunisés devenus porteurs de germes et les seconds comme des réfractaires indemnes de virus.'*

If we take his words for what they mean today, it would seem that Bruynoghe saw the adaptation of the bacterium to the phage as if they possessed a defense mechanism keeping the phage in check.

The term ‘refractory’ which he used to characterize the completely resistant bacteria leaves us in doubt whether he saw these altered bacteria as ‘newly induced’ or as selected descendants from variants that preexisted the arrival of the phage (Lamarckian versus Darwinian evolution).

Unfortunately, neither Bordet nor Bruynoghe explicitly asked themselves these questions. This was true for most bacteriologists of the 1920s and 1930s: ability of bacteria to rapidly change their hereditary characters was simply accepted as a matter of fact. As a result, bacterial geneticists of the 1940s accused them of blindly adhering to a Lamarckian view of evolution.

Bruynoghe and Bordet both discontinued their bacteriophage work in the mid-1920s. However, Bordet continued defending his theory until at least the early 1930s. The first electron micrographs of bacteriophages were obtained in 1940 and definitively endorsed the viral hypothesis.



Richard Bruynoghe (left) and Jules Bordet (right) around 1920

Alfons Billiau, Rega Institute, University of Leuven.

Reference List

1. Bordet, J. and M. Ciuca. 1920. Le bactériophage de d'Herelle, sa production et son interprétation. *C.R.Soc.Biol.(Belgique)* 83:164-166.
2. Bruynoghe, R. 1923. Contribution à l'étude de la nature des bactériophages. *Bull Acad R Méd Belg* 3:360-371.
3. Maisin, J. and R. Bruynoghe. 1921. Au sujet de la nature du principe bactériophage - I. *C.R.Soc.Biol.(Belgique)* 84:467-468.
4. Maisin, J. and R. Bruynoghe. 1921. Adaptation du bactériophage. *C.R.Soc.Biol.(Belgique)* 84:468-470.
5. Bruynoghe, R. 1921. Au sujet de la nature du principe bactériophage - II. *C.R.Soc.Biol.(Belgique)* 85:258-260.
6. Brutsaert, P. 1923. La virulence des bactériophages. *C.R.Soc.Biol.(Belgique)* 89:87-90.
7. Bruynoghe, R. and R. Appelmans. 1922. La neutralisation des bactériophages de provenance différente. *C.R.Soc.Biol.(Belgique)* 86:96-98.
8. Maisin, J. and R. Bruynoghe. 1921. Au sujet du principe bactériophage et des anticorps. *C.R.Soc.Biol.(Belgique)* 84:755-756.
9. Appelmans, R. and R. Bruynoghe. 1921. Le dosage du bactériophage. *C.R.Soc.Biol.(Belgique)* 85:1098-1099.
10. Wagemans, J. and R. Bruynoghe. 1922. Au sujet de la constitution du bactériophage. *C.R.Soc.Biol.(Belgique)* 87:1244-1245.
11. Bordet, J. and M. Ciuca. 1921. Evolution des cultures de *coli* lysogène. *C.R.Soc.Biol.(Belgique)* 84:747-748.
12. Bruynoghe, R. and J. Maisin. 1921. Au sujet des microbes devenus résistants au principe bactériophage. *C.R.Soc.Biol.(Belgique)* 84:847-848.

Belgian Society for Microbiology

Recent microbiology highlights

Shipworm endosymbionts and bio-fuel production. Shipworms are marine bivalve mollusks that drill tunnels in submerged wood. The ability of *Bankia setacea* to feed on wood derives from specialized enzymes produced by endosymbiont bacteria, including *Teredinibacter turnerae*. Intriguingly, the gut does not contain microbiota. The symbiont lives as a simple community in specialized cells (bacteriocytes) in the gills (forming an organ known as Deshayes' gland) and the enzymes are secreted and transported to the gut where they can do their work without competition from other bacteria. Because only the wood-degrading enzymes and no other bacterial proteins are transported to the gut, the system may help to define which enzymes are minimally necessary to digest wood, a key question in research on bio-fuel production. (O'Connor,R.M. et al. *Proc. Natl. Acad Sci U. S. A.* 2014, 111:E5096-E5104.)

An ancestral virus suitable as vehicle in gene therapy. Adeno-associated viruses (AAVs) are small DNA viruses that can only replicate when assisted by adenoviruses. They are of no significance as disease agents but show promise as vehicles in gene therapy. However, many individuals are immune to some AAVs due to previous natural infections. Investigators have used various approaches to obtain AAVs that evade pre-existing immunity. One recent strategy has consisted in defining the evolutionary history of current AAVs and engineering a putative ancestral AAV. Tests in mice showed that the virus was suitable to introduce a transgene despite the presence of antibodies against some current AAVs. (Zinn, E. et al. Cell Reports 2015, 12:1056-1068; Taylor, A.P. The Scientist 2015, July 31).

More about interaction between gut bacteria and viruses (see previous newsletter). Human noroviruses (*Caliciviridae*), responsible for frequent outbreaks of acute gastroenteritis can in some patients also cause persistent infection. In a mouse model, treatment with antibiotics prevented persistent infection with mouse norovirus. The effect depended on the presence of an intact receptor for interferon-l (IFN-l). The study shows that the composition of the enteric microbiome critically affects the ability of noroviruses to establish and maintain persistent infection. In a separate study, treatment with IFN-l was found to cure mice from persistent norovirus infection. The effects were independent from adaptive immunity, showing the ability of innate immunity to achieve complete elimination of the virus. (Baldridge,M.T. et al. *Science* 2015, 347:266-269; Nice,T.J. et al. *Science* 2015, 347:269-273.)

A mechanism by which commensals in the gut flora escape innate host defense. Commensal *Bacteroidetes* were shown to possess a gene, *lpxF,* that codes for a phosphatase that clips off a phosphate group from the organisms' LPS, thereby making it less susceptible to be targeted by positively charged antimicrobial peptides secreted by the host. Deletion of the gene was found to result in loss of the *Bacteroides*'s ability to compete with pathogens *in vivo*. Among genes of commensals accounting for resistance to innate immunity, *lpxF* is shared by many species.(Cullen, T.W. et al. *Science* 2015, 347: 170-75; Yandell, K. *The Scientist* 2015, January 8.)

Urban sewage effluent, the human microbiome and obesity. A study in the U.S. has revealed a correlation between the distribution of fecal microorganisms such as *Bacteroidaceae* in municipal sewage and the prevalence of obesity. The investigators used oligotyping of 16S rRNA gene sequence data to compare the bacterial distribution in a stool data set to a sewage influent data set from 71 U.S. cities. Fecal bacterial communities were less diverse in sewage than in individual stool samples but their distribution patterns reflected human population variation and predicted whether samples represented lean or obese populations with 81 to 89% accuracy. (Newton,R.J. et al. 2015, *MBio.* 6:e02574-14; Akst, J. *The Scientist,* 2015 march 10).

New legislation for the use of microbial (and other) resources for studies and applications - *The Nagoya Protocol*

The Nagoya Protocol on 'Access to Genetic Resources and the Fair and Equitable Sharing for Their Utilization' was adopted in 2014 and will have impact on all researchers working with biological resources. It is a legal binding instrument for the implementation of the Convention on Biological Diversity (CBD). The CBD has three main goals i) the conservation of biological diversity; ii) the sustainable use of its components and iii) the fair and equitable sharing of benefits arising from its utilization. Basically the CBD recognises the sovereign rights of countries over their own biological resources and the so-called genetic resources contained therein. Access to genetic resources in a country that is party to the CBD (i.e a country that has signed and ratified the CBD) requires prior informed content (PIC) from the competent authority in that country and a setting of mutually agreed terms (MAT) between provider and user. Parties are free to decide whether access to genetic resources is subject to such requirements or not. But the Party must assure that, within their territory, genetic resource, originating from other Parties (countries) are handled according the CBD and that benefits arising from the use of the genetic resources or traditional knowledge associated with these resources are shared fairly and equitable.

The Nagoya Protocol aims to provide guidance for the parties to implement the CBD's fair and equitable sharing of benefits arising from the utilization of the resources by adopting their national access and benefit sharing (ABS) legislation. The biological material (including DNA extracts) held in collections in covered by the Nagoya Protocol. This Protocol is now going to be implemented in the EU [1] and guidelines are being prepared and are currently under consideration by different groups. The objective at European level is an implementation of the Nagoya Protocol on the 12th of October 2015.

[1] For more information see regulation (EU) No511/2014.(http://eur-lex.eruopa.eu/legal-content/EN/EN/TXT/?uri=celex:32014R0511)

It should be noted that this legislation seems to create conflict with obligations under the IPPC, for example it is not clear if specimens isolated from imported consignments can be stored and used for diagnostic and research purposes. This needs to be clarified.

On 13 October a training workshop around practical implications on ABS is organised in Brussels. It is the aim of the workshop to provide information for users of microbial resources on the EU ABS regulation in a practical way in their everyday work. The participants will have the occasion to test their practical knowledge on the application of the ABS regulation with interactive case studies base on real live situations and realistic examples and scenarios.

By this the participants will better understand the application and obligations of the new ABS regulation and which steps they need to follow and which practical measures they should take while using genetic resources that originate from Parties of the CBD and the Nagoya Protocol. The workshops targets both academics and scientists involved in Research and Development activities.

Evolution on the application of ABS in the frame of the Nagoya Protocol will be reported in our following news letters.

Interesting websites and video's: https://www.youtube.com/watch?v=GbYmsi9ShP0 and http://www.biodiv.be/biodiversity/about_biodiv; https://www.cbd.int/abs/ and http://www.biodiv.be/biodiversity/about_biodiv

Paul De Vos
Lab of Microbiology
Ghent University

Belgian Society for Microbiology

Hans Christian Gram and the Gram-staining: from fame to shame?

Rise and decline of the importance of the Gram-staining

The Gram-staining – named after its inventor Hans Christian Gram (1853-1938), a Danish medical doctor and bacteriologist – remains fixed in the memories of many generations of students …and of their professors. It was (and still is) a cheap, simple and quick staining technique that makes bacteria (and yeasts) in culture fluids, tissues, smears,… easily visible under the microscope. It also appeared to allow to differentiate roughly between two large groups of bacteria, depending on the outcome of the staining procedure: the Gram-positive ones (colored purple-blue) and the Gram-negative ones (colored pink-reddish). After staining with a basic purple dye (typically crystal violet), iodine is added as a mordant, a chemical that helps retain the stain in certain cells (the Gram-positive ones) but not in others ; those cell structures (of Gram-negative cells) that cannot retain crystal violet are then decolorized by the subsequent treatment with 95% ethanol. At this stage, these cells look as unstained under the microscope. Only after counter-staining with a different-colored basic red dye (typically safranin or fuchsin), the two cell types can be easily distinguished microscopically by their different colors. This counter-staining step was actually added on to Gram's staining a few years later by C. Weigert, a German pathologist.

We know now that the Gram-staining differentiates bacteria by their difference in the chemical and physical properties of their cell walls, especially related to the peptidoglycan component, present as a tick multilayered and important wall-layer in the Gram-positive bacteria, and being thin and a minor layer in the Gram-negatives ones.

It is believed that once inside the cells, the crystal violet and the iodine combine to form a large complex CV-I; this complex cannot be washed out of the intact peptidoglycan layer of Gram-positive cells by alcohol; in Gram-negative bacterial cells, the alcohol wash disrupts the outer lipopolysaccharide layer and the CV-I complex is washed out from the thin layer of peptidoglycan. The exact molecular mechanism of the differential Gram-stain is still today a partial mystery! Soon it was realized that many bacteria do not respond to this staining technique, such as the Archaea, Mycoplasma, capsulated bacteria, …. (called Gram-nonreactive cells) and that the outcome is also dependent on the (culture) age of the bacteria under study (named Gram-variable cells). Nevertheless, it used to be almost always the first step in the identification procedures of a bacterial isolate, whether in the food, medical, industrial or environmental sectors. It still is a universal and very practical staining and microscopical test for generations of young microbiology students to practice their hands-on skills in the microbiology lab!

Although nowadays still in use – but now as one of the very many identification and diagnostic tools in food, medical, industrial and environmental settings, it has lost its glamour and strict bacterial differentiating power. However, it remains an effective, cheap, and rapid diagnostic medical tool (still very important in the third world medical care!), when infections are suspected, since Gram-stain results (on body fluids, autopsy-, biopsy-samples,..) are much more quickly available than culture results. Today it has generally been largely superseded by molecular nucleic acid diagnostic techniques, such as PCR and other modern gene-sequencing methodologies, that are far more specific and informative …and expensive than differential staining.

Students prefer to boast being able to use PCR techniques, while Gram-staining make them feel a bit ashamed as being old fashioned! Nevertheless its inventor Hans Christian Gram gained international fame and acclaim after he published his method in 1884 at the age of 31! It was soon taken up in the laboratories of the then famous German bacteriologists, such as Robert Koch, Carl Weigert (whom improved it by introducing the counter-stain step) and Friedrich Löffler,.. and from there it spread all over Europe, and it is - depending on the context - after >125 years still going strong!

2. The birth of the Gram-staining

H.C. Gram studied initially botany at the University of Copenhagen and his interest in plants introduced him to the fundamentals of pharmacology and to the use of the microscope, then thé scientific instrument. In 1878 he switched to medical school and graduated cum laude in 1883 ; he received his university's "Gold Medal" for his haematological research. About that time, Carl Julius Salomonsen (1847-1924) was appointed lecturer in bacteriology with the specific task to run courses in practical bacteriological techniques for medical students and H.C. Gram was one of the first students to enroll.

Upon finishing the course, Prof. C.J. Salomonsen introduced H.C. Gram – who had expressed his eagerness to travel and study abroad – to Prof. Carl Friedlander, pathologist at the municipal hospital Friedrichshavn in Berlin, Germany. He was renown for his studies on the etiology of lobar pneumonia and was also founder and editor of the journal "Fortschritte der Medizin". H.C. Gram arrived in C. Friedlander's lab on 22 October 1883 and worked there till 20 March 1884. While working in the morgue of the city hospital, he devised and optimized his staining technique in November-December 1883, actually not to distinguish one type of bacteria from another, but to make them better visible in stained sections of human and animal lung tissue, especially to visualize pneumonia-cocci, while the other tissue elements remain unstained.

He demonstrated also the usefulness of his stain method for other bacteria ("Schizomycetes") and other tissues; he also observed that certain bacterial cells in his lung tissues (most probably *Klebsiella* sp.) and "capsulated" strains did not stain at all, since he did not apply the counter-stain. His landmark and "one only"- publication of 5 pages on the topic appeared on 15 March 1884 in Fortschr.Med.

3. Gram's own opinion about his Gram-staining

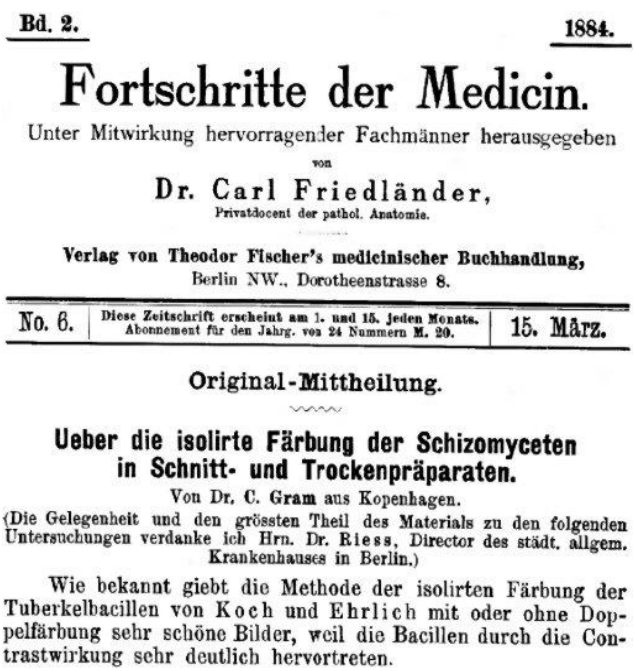
It has long been a matter of debate as to what extent H.C. Gram realized the significance of the staining method that he had devised while he was in C. Friedlander's lab. H.C. Gram seemed to be indeed a modest scientist as he wrote at the end of his paper : "Studies on Schizomycetes have been significantly improved by the use of this method. It is because of this that I publish my results, although I am well aware that they are brief and with many gaps. It is to be hoped that this method will also be useful in the hands of other workers". We all know the outcome of his prophecy! However other writings of H.C. Gram indicate that he cherished and valued his "invention" very much. While working in Berlin, H.C. Gram corresponded regularly by letter with his Prof. C.J. Salomonsen in Copenhagen. These letters (in Danish) were kept (and were largely forgotten) in the Department of Manuscripts, Royal Library, Copenhagen. Around 1980 several of these "Gram to Salomonsen" - letters were systematically restudied "against history" and they revealed that H.C. Gram really grasped the significance of his method : he used terminology such as "..what seems to be a very good method to stain the cocci, while the tissue and the cell nuclei remain unstained. …", "… my excellent iodine method … " and "…my epoch making discovery… "! He was as estatic in his private letters as he was modest in his scientific paper : he indeed refrained from being outspoken in his 1884 publication, ….since he did not want to surpass his Berlin host, Prof. C. Friedlander!

4. Fame, but no shame!

After his short stay in Berlin, C.H. Gram spent several weeks holiday in Northern Italy and Switzerland, and then went to the University of Strassbourg to work on digitoxin, the heart glycoside from the foxglove-plant *Digitalis purpurea*. He returned beginning August 1884 back to Copenhagen, where he spent the rest of his career. By now his staining method became well known as it had been presented by Prof. C.Friedlander at the 3rd Congress of International Medicine in Berlin (21-24 April 1884) and at the International Medical Congress in Copenhagen in August 1884 by H.C. Gram himself. His lecture text, entitled "Ueber die Färbung der Schizomyceten in Schnittpräparaten" appeared in 1886 in the "Compte-Rendu" of the Copenhagen Congress. In 1891, H.C. Gram became a lecturer in pharmacology and was later that year appointed professor at the University of Copenhagen. In 1900 he resigned this chair to become a professor of Medicine, publishing over the years four volumes of clinical lectures, widely used in Denmark.

He retired from the university in 1923 at the age of 70. He died in 1938.

His Gram-staining method survived him for many decades to come, but indeed lately it has lost part of its fame, but for shame …. there is no room and no need!



Bd. 2. 1884.

Fortschritte der Medicin.

Unter Mitwirkung hervorragender Fachmänner herausgegeben von

Dr. Carl Friedländer,
Privatdocent der pathol. Anatomie.

Verlag von Theodor Fischer's medicinischer Buchhandlung,
Berlin NW., Dorotheenstrasse 8.

No. 6. | Diese Zeitschrift erscheint am 1. und 15. jeden Monats. Abonnement für den Jahrg. von 24 Nummern M. 20. | 15. März.

Original-Mittheilung.

Ueber die isolirte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten.

Von Dr. C. Gram aus Kopenhagen.

(Die Gelegenheit und den grössten Theil des Materials zu den folgenden Untersuchungen verdanke ich Hrn. Dr. Riess, Director des städt. allgem. Krankenhauses in Berlin.)

Wie bekannt giebt die Methode der isolirten Färbung der Tuberkelbacillen von Koch und Ehrlich mit oder ohne Doppelfärbung sehr schöne Bilder, weil die Bacillen durch die Contrastwirkung sehr deutlich hervortreten.

Hans Christian Gram (1853-1938) and his famous paper on the Gram-staining

5. Literature

-Austrian, R. 1960. The Gram stain and the etiology of lobar pneumonia, an historical note. Bacteriol. Rev., 24 (3),261-265
-Barken, K.B., Haagensen, J.A.J., Tolker-Nielsen,T. 2007. Advances in nucleic acid-based diagnostics of bacterial infections. Clin. Chim. Acta, 384(1-2), 1-11
-Gram, C. 1884. Ueber die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten. Fortschr. Med., 2, 185-189
-Jacobson, W. 1983. Gram's discovery of his staining technique. J. Infection, 7, 97-101
-Lautrop, H. 1981. Christian Gram on the Gram Stain in Letters to Carl Julius Salomonsen, 1883-1884. ASM News, 47(2), 44-49
-Malorny, B., Tassios, P.T., Radström, P., Cook, N., Wagner, M., Hoorfar, J.(2003). Standardization of diagnostic PCR for the detection of foodborne pathogens.Int. J.Food Microbiol., 83(1), 39-48
-Sogaard, M., Norgaard, M., Schonheyder, H. 2007. First notification of positive blood cultures: high accuracy of the Gram stain report. J.Clin.Microbiol., 45(4), 1113-1117

Em. Prof. dr. ir. Erick Vandamme, Dept. Biochemical and Microbial Technology, Fac. Bioscience Engineering, Ghent University; erick.vandamme@UGent.be

ESGB
ESCMID STUDY GROUP FOR BIOFILMS
European Society of Clinical Microbiology and Infectious Diseases

EPASG
ESCMID PK/PD OF ANTI-INFECTIVES STUDY GROUP
European Society of Clinical Microbiology and Infectious Diseases

Supported by FWO-Vlaanderen, Ghent University and BELSPO-IUAP program,

Antimicrobial resistance in microbial biofilms and options for treatment

Ghent, Belgium, October 5-7, 2016

Organising Committee: Tom Coenye & Françoise Van Bambeke

International Scientific Advisory Board: Thomas Bjarnsholt (DK), William Couet (FR), Veronika Hola (CZ), Christine Imbert (FR), Elisabeth Nielsen (SWE), Antonio Oliver (ESP), Jason Roberts (AUS), Ursula Theuretzbacher (AT), Craig Williams (UK)

Conference Venue
Ghent University Culture and Convention Centre "Het Pand", Onderbergen, Ghent, Belgium



Topics that will be addressed include

- Evolution of antimicrobial resistance in biofilms
- Models to study resistance, tolerance & treatment *in vitro* and *in vivo*
- Modified medical devices
- Novel antibiotics with increased anti-biofilm activity
- Novel approaches to drug delivery
- PK/PD in biofilm infections
- Quorum sensing inhibition
- Potentiating the anti-biofilm activity of antibiotics
- Targeting the matrix as a novel approach to treatment
- Tolerance mechanisms is biofilms
- …

Sessions will take place from 5 October (afternoon) until 7 October (early afternoon)

Registration & Hotel Accommodation
Information on the registration and hotel booking can be found on the conference website

Abstract Submission
Deadline for abstracts for poster and oral sessions: 15 May 2016. Guidelines for submission are available on the congress website.

Registration Fees include opening reception, coffee breaks and sandwich lunches

Registration: early bird until 15 July 2016

www.biofilmresistance.be

http://www.biofilmresistance.be

Composition of the BSM Board

President: Jozef Anné (KU Leuven)
Secretary & representative in the IUMS : Paul De Vos (UGent)
Treasurer & liaison with NVVM : Tom Coenye (UGent)
FEMS delegate: Paul Cos (UAntwerpen)

Members : Spiros Agathos (UCL), Alfons Billiau (KU Leuven), Guy Cornelis (UNamur), Pierre Cornelis (VUB, liaison officer ASM), Paul Cos (UAntwerpen, FEMS delegate), Herman Favoreel (UGent), Isabelle George (ULB), David Gillan (UMons), Laurent Gillet (ULg), Natalie Leys (SCK-CEN), Max Mergeay (SCK-CEN), Dominique Schols (KU Leuven), Jos Vanderleyden (KU Leuven)

Contributed to this issue: Jozef Anné, Alfons Billiau, Tom Coenye, Paul De Vos, Erick Vandamme

Call for contributions

With this quarterly newsletter the BSM board wants to improve its communication with BSM members and we hope to bring you useful microbiology-related information on a regular basis.

Of course this is only possible with your contributions and we would like to invite you to submit these contributions to BSM.newsletter@gmail.com (preferably as a Word document).

What can you submit ? Basically anything that is microbiology-related : vacancies in your lab, announcements of seminars, a summary of important/interesting research findings etc. If you want to discuss whether something would be suitable for inclusion in the newsletter prior to preparing the text, feel free to contact us as well.

VISIT US AT : http://www.belsocmicrobio.be/