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Louis Pasteur and the birth of microbiology in Denmark

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Høiby N. Louis Pasteur and the birth of microbiology in Denmark. APMIS 2022. Louis Pasteur's work initiated the birth of microbiology in Denmark. Carl Julius Salomonsen was the pioneer who inspired and taught Christian Gram, Thorvald Madsen and Bernhard Bang bacteriological techniques in his annual bacteriological course which he started in 1883 at the University of Copenhagen. These pioneers developed Danish human and veterinary microbiology and became world famous. Emil Chr. Hansen developed Danish technical/industrial microbiology in the Carlsberg Laboratory and purified yeast and designed equipment for the propagation of pure yeast which was used worldwide in beer brewing. He also became world famous. The fascinating birth and development of Danish microbiology is summarized in this article, which is dedicated to the 200th birthday of Louis Pasteur, December 27, and 100th birthday of the Danish Pasteur Society, October 25, 2022.

Key words: Bacteriology; epidemiology; microbiology; Pasteur; veterinary microbiology.

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INTRODUCTION

Anthonie van Leeuwenhoek from Delf (1632–1723) invented the microscope and discovered bacteria in 1674 and made drawings of these 'very little animalcules' in fresh water with different morphology [1]. Otto Friedrich Müller (1730–1784) was the first bacteriologist in Denmark and the first systematic bacteriologist in the world [2]. He described 6 species in the genus *Vibrio* which became the basis of systematic bacteriology for the following 100 years. They were described in 1773–74 in his book '*Vermium terrestrium et fluviatilium historia*' and in his next book '*Animalcula infusoria'* from 1786 [2]. Since his work was the beginning of bacterial taxonomy, he was celebrated by being cited on the front page of International Journal of Systematic Bacteriology, the official publication of the International Committee for Systematic Bacteriology: '*... the sure and definite determination (of species of bacteria) requires so much time, so much acumen of eye and judgment, so much perseverance and patience, that there is hardly anything else so difficult - Mueller*'. This happened 100 years after Leeuwenhoek from Delft and 48 years before the birth of Louis Pasteur (1822–1895) and 81 years before Pasteur's experiments which disproved spontaneous generation (1860–66) [3] and his study of fermentation [4] and diseases of silkworms (1870) [5], wine (1867) [6], beer (1876) [7] and pasteurization [6] (1865) and subsequently his study of virulent infections and protective immunization with attenuated microbes (1877–1885) [8]. Interestingly, however, the Danish fairy tale author Hans Christian Andersen (1805–1875), had seen the small microorganisms when he, in 1830, visited the Danish botanist Niels Hofman-Bang (1776–1855) who showed him a microscope [9–11]. H.C. Andersen discussed the microorganisms with his friend and mentor the physicist who discovered electromagnetism H.C. Ørsted (1777–1851) and wrote the fairy tale 'The water drop' in 1847 where he compared the motility of the microorganisms with busy people in a large city.

The beginning of microbiology, therefore, was indeed very slow, but with the pioneer contributions of the chemist Louis Pasteur, who connected microbes with diseases in industrial products (silk, beer and wine), in animals and in humans and founded the new revolutionary research area which was microbiology. The Golden Age of Microbiology (1850–1914) began with Pasteur's work

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(Table 1). The other great pioneer was the German physician Robert Koch (1843–1910) who in 1876 described *Bacillus anthracis* as the cause of anthrax in animals and humans and thereby introduced his later so famous Koch's criteria as proof of the ethiology of a clinical characteristic infection, initiating the birth of clinical microbiology [12]. This period also included the description of antiseptic surgery and disinfection of infected wounds in 1867 by Joseph Lister (1827–1912) who was inspired by Pasteur's germ theory [13,14] and used carbolic acid and of Koch's work on the activity of carbolic acid and other disinfectants, hot air and steam on *B. anthracis* spores and *Micrococcus prodigiosus* [15–18].

The first Danish bacteriological paper after Müllers books was published by the botanist and ecologist Eugen Warming (1841–1924) in 1875–76 who described bacteria at the Danish coastal water but he did not continue his bacteriological work [20,21]. The bacteriological pioneer in Denmark was, however, Carl Julius Salomonsen [20] who graduated as a medical doctor in 1871 together with 45 other candidates. He became employed at Kommunehospitalet in Copenhagen 1873-74 where he did his first bacteriological diagnosis. He found chains of coccal bacteria in an aspirate from the infected knee of a young carpenter. Salomonsen injected the aspirate into the peritoneum of a rabbit, which succumbed due to the infection and Salomonsen found the same bacteria in the rabbit. He injected these bacteria into a new rabbit which also died and he could again find the same bacteria in the inflamed tissue. Remarkably, therefore, Salomonsen already used the later so famous Koch's postulates [22] to prove that the bacteria were causing the infection. Salomonsen's first scientific article was published in 1876 in a botanical journal and described the isolation of different bacteria [23]. Already around 1880, the development of bacteriology in Denmark became divided between the medical branch (Salomonsen), the technical/industrial branch (Emil Christian Hansen, 1842–1909) and the veterinary/food branch (Bernhard Bang, 1848–1932) [20] who all were born in the period 1842–48 and all became world famous scientists.

THE DEVELOPMENT OF MEDICAL MICROBIOLOGY IN DENMARK

Carl Julius Salomonsen

Salomonsen worked on his doctoral thesis 'Putrifaction of Blood' in the biomedical laboratory of professor of physiology Peter Ludvig Panum (1820–1885). Just after his dissertation in 1877, Salomonsen traveled to Breslau, Prague and Paris. In Breslau, he participated in Julius F. Cohnheim's (1839–1884) famous course of experimental pathology and met Robert Koch [20]. Cohnheim and Salomonsen introduced tuberculosis in the anterior chamber of a rabbit's eye in 1877 and thereby proved, that tuberculosis was a transmissible disease. These results inspired Koch to search for the cause of tuberculosis which he succeeded in 1882. In Prague, Salomonsen visited Edwin Klebs (1834–1913), whereas Pasteur was on summer vacation when Salomonsen arrived to Paris. However, Salomonsen later visited Pasteur in 1880, 1887 and 1890 and visited Koch in Berlin in 1882. Salomonsen was appointed docent of bacteriology in 1883 at the University of Copenhagen which was the first University chair of bacteriology in the world [22] and he organized his first laboratory course of bacteriology at the university the same year with 11 participants two of whom (Fig. 1, Table 1) later became world famous (Christian Gram and Bernhard Bang) [22]. Two years later (1885), he published a textbook 'Bakteriologisk Teknik for Medicinere' which was printed in 3 editions (1885–1894) and was translated into English, French, Russian and Spanish languages.

Thorvald Madsen

One of Koch's collaborators, Emil A. von Behring (1854–1917) together with his Japanese colleague S. Kitasato (1853–1931) had shown in 1890 that animals inoculated with *C. diphtheria* became resistant to reinfection due to the production of antibodies against the diphtheria toxin. These experiments were the birth of serum therapy and Behring received the first Nobel Prize in 1901 for his work [25]. There was opposition against the results from the famous professor Rudolf Virchow (1821–1902) but Emile Roux (1853–1933) from the Pasteur Institute, who had detected the diphtheria toxin in 1889, reported at the 10. International Congress of Hygiene in Budapest 1894 about the excellent results obtained at a diphtheria hospital in Paris where they used the new serum therapy [24]. Salomonsen who was appointed professor at the University of Copenhagen in 1893 traveled to the Pasteur Institute in 1894 and learned how to produce horse antiserum against diphtheria. He began to produce anti-diphtheria toxin in 1894 by immunization of horses together with his assistant Johannes Fibiger (1867–1928) and subsequently the newly graduated medical doctor Thorvald Madsen (1870–1957), who had participated in Salomonsen's course of bacteriology where he was recruited by Salomonsen [26]. The antiserum was donated for free to

Table 1. Louis Pasteur' legacy: The discovery of important causative bacteria of infections in the Golden age of Microbiology1850–1914 and the concurrent influence on the birth of Danish bacteriology. (modified from [19])

Year	Name of scientist	Discovery/event
1857	Louis Pasteur	Fermentation
1859	Louis Pasteur	Disprove spontanous generation, germ theory
1864	Louis Pasteur	Pasteurization
1867	Joseph Lister	Antiseptic surgery
1873	Amauer Hansen	Leprosy, *Mycobacterium leprae*
1876	Robert Koch	Germ theory of diseases
1877	Robert Koch	Antrax, *Bacillus anthracis*
1877[7]	Carl Julius Salomonsen	Studies of decay of blood (thesis)
1878	Robert Koch	Suppuration, *Staphylococcus*
1879	Albert Neisser	*Neisseria gonorrhoe*ae
1880	Carl J. Eberth	*Salmonella typhi*
1880	Louis Pasteur	Attenuated vaccines, fowl cholera, antrax
1881	Robert Koch	Aseptic, disinfection by hot air and steam
1881	Robert Koch	Pure culture
1881	A. Ogston	Suppuration, *Streptococcu*s
1882	Robert Koch	*Mycobacterium tuberculosis*
1882[7]	Emil Chr. Hansen	Pure culture of *S. cerevisiae*[1]
1883	Robert Koch	*Vibrio cholerae*
1883	T. Klebs, F. Loeffler	*Corynebacterium diphteriae*
1883[7]	C.J. Salomonsen	Docent at Copenhagen University[2]
1883[7]	C.J. Salomonsen	First laboratory course of bacteriology[3]
1984	Robert Koch, F.Loeffler	Koch's postulates published
1884[7]	Christian Gram	Gram staining method
1884	A. Nicholaier	Tetanus, *Clostridium tetani*
1885	Loius Pasteur	Rabies vaccination of Joseph Meister
1886	T. Escherich	*Escherichia coli*, digestion physiology
1886	A. Fraenkel	Pneumonia, *Streptococcus pneumoniae*
1887	A. Weischelbaum	Meningitis, *Neisseria meningitidis*
1888	A. Gaertner	Gastroenteritis, *Salmonella enteritidis*
1890	E. von Behring	Diphtheria antitoxin
1890	P. Ehrlich	Theory of immunity
1892	W. H. Welsch	Gas gangrene, *Clostridium perfringens*
1894	A. Yersin, S. Kitasato	Plague, *Yersinia pestis*
1896	E. van Ermengem	Botulism, *Clostridium botulinum*
1898[7]	J. Fibiger	RCT[8] with anti-diphtheria toxin[4]
1897[7]	Bernhard Bang	*Bacillus (Brucella) abortus*
1898	K. Shiga	*Shigella dysenteriae*
1900	H. Schottmüller	*Salmonella paratypi*
1902[7]	C.J. Salomonsen	Statens Seruminstiut was established[5]
1903	F. Schaudin, E. Hoffmann	Syphilis, *Treponema pallidum*
1906	J. Bordet, O. Gengou	Whooping cough, *Bordetella pertussis*
1906[7]	Bernhard Bang	Paratuberculosis, *M. paratuberculosis*[6]
1914	R. Inada, Y. Ide	Leptospirosis, *Leptospira interrogans*

[1]Beer disease was due to contamination of *S. cerevisiae* wih 'wild' yeast.
[2]The first university chair of bacteriology in the world.
[3]Eleven participants among them were Bernhard Bang and Christian Gram.
[4]C.J. Salomonsen and his assistant Thorvald Madsen (1870–1957) produced the horse-anti-diphteria toxin serum at the university for the trial.
[5]Thorvald Madsen was the daily leader and became its director 1909–1940.
[6]*M. avium* ssp. paratuberculosis.
[7]Danish bacteriologists.
[8]Randomized Controlled Trial.

Danish physicians to treat patients with diphtheria. Salomonsen received a 10,000 DKK grant (equal to 800,000 DKK = 107,000 € in 2022 money) from the Danish government 1895–96 and later 10,000 DKK from a private foundation. Since it was difficult to induce a sufficiently high concentration of antibodies against diphtheria toxin, Thorvald Madsen performed a number of experiments to establish the optimal conditions for the production of antibodies in horses which resulted in his thesis (1896) 'Experimental research of the Diphtheria toxin'. He visited Paul Ehrlich (1854–1915, who received the

Fig. 1. Professor Carl Julius Salomonsen (1847–1924) (right, white coat) teaching at his course of medical microbiology, Knud Faber (1862–1946) (standing to the left, he became professor of medicine) and Johannes Fibiger (1867–1928) (center, blue coat, he became professor of anatomical pathology and received the Nobel prize in physiology or medicine 1926). Painting by Tycho Jessen, the painting is at the Nationalhistorical museum, Frederiksborg Castle, Hillerød.

Nobel Prize 1908) and learned about detoxified toxins (toxoids) which were still immunogenic as described in Ehrlichs publication 'Die Werthbemessung des Diphtherieheilserums und derem theoretische Grundlagen'. (Klinisches Jahrbuch 6: 299–326; 1897). Thorvald Madsen also started collaborating with the Swedish chemist Svante Arrhenius (1859–1927), who received the Nobel Prize in 1903. The first samples of anti-diphtheria serum were used to treat patients at Blegdamshospitalet already in June 1895. The head of the hospital Professor Søren Th. Sørensen had previously used samples of German antiserum and French antiserum to treat his patients with somewhat disappointing results. Johannes Fibiger (he graduated as a physician in 1890 and visited Koch in 1891, and he received the Nobel Prize in 1927) convinced Sørensen to organize a statistical optimal clinical experiment of the effect of Salomonsen's horse antiserum against diphtheria toxin. Fibiger's thesis from 1895 was carried out in Salomonsen's university institute and the title was 'Bacteriological studies of Diphtheria'. The experiment [27], which became the world's first randomized, controlled experiment, began on May 13, 1896, and ended one year later; 239 patients admitted on even dates received antiserum subcutaneously and 245 patients admitted on odd dates were not treated with antiserum (controls). The patients were clinically subdivided in those with croup (barking coughing) and those without croup. The results were statistically significant, since 8 treated patients died whereas 30 controls died ($p < 0.001$) and the same results were found in the subgroups with or without croup. Fibiger explained that his convincing result was due to higher doses of antiserum and better methods of carry out the clinical experiment. After the results were published [24], the demand of antiserum increased and 5036 doses were produced in 1900 and 6305 in 1901, which, however, could not cover the clinical demand in Denmark. Salomonsen had political connections and his assistant Thorvald Madsen was son of general Vilhelm H.O. Madsen who at that time was minister of war in Denmark [28,29]. Salomonsen and Thorvald Madsen had already in 1898 found an area with military barracks which would be suitable for establishment of an Institute for serotherapy, but the university was not willing to house a factory which it thought should rather be part of the public health system. A law of March 22, 1901, was approved by the Danish Folketinget with the purpose to establish an institute which should produce anti-diphtheria serum: the Statens Seruminstitut, which was opened in 1902 [28]. Salomonsen became the first director and as daily leader was appointed Thorvald Madsen who in 1909 became the second director of the institute (1909–1940), when Salomonsen became Rektor (president) of the University of Copenhagen (1909–1910) [24]. Salomonsen retired in 1920. Salomonsen's own university Institute of General Pathology moved to new buildings (1910) at Rigshospitalet. It was the first institute of general pathology in Europe which was located within a hospital in order to facilitate the collaboration between microbiologists and clinicians. The subsequent development of Danish microbiology took place at the Statens Seruminstitute where Thorvald Madsen was an eminent leader who recruited Oluf Thomsen (1878–1940) as daily head of diagnostic bacteriology. Oluf Thomsen was followed by Martin Kristensen (1888–1984) when he succeeded Salomonsen in 1920 as professor at the University of Copenhagen [26]. Thorvald Madsen expanded the purpose of the institute from a factory producing antiserum to also be responsible for the production of vaccines in Denmark, for comprehensive diagnostic work of bacterial, viral, mycological and parasitological infections including serological analyses and hormone analyses and to produce blood plasma. Thorvald Madsen was very interested in epidemiology and he persuaded the Rockefeller Foundation to donate money for buildings and a for an epidemiological tuberculosis study, since Denmark was an unique field laboratory for such epidemiological investigations. K.A. Jensen (1894–1971) was in charge of the tuberculosis research until he became professor of Microbiology at University of Copenhagen in 1940 [30]. Thorvald Madsen became president of the League of Nations's Health Committee (which became replaced by WHO after the 2nd World War) in 1921 and established its Commission on Biological Standardization chaired by himself and with the British sir Henry Dale as vice-chairman and Statens Seruminstitut became the center for standardization of biological products [28]. Thorvald Madsen had been able to develop Statens Seruminstitut to one of the leading microbiological research institutes in the world during his leadership in 1909–1940. His strategy had been to recruit medical doctors to basic research at the institute where he gave them excellent working conditions and economy for their research, and this strategy was very successful. When he retired in 1940, his successor Jeppe Ørskov (1892–1977) continued to expand the institute and started production of polio vaccine (Salk) which was approved for use on April 27, 1955, 14 days later than the original American vaccine (Salk) was approved in in the USA and these two countries were the only countries in the world which could produce polio vaccine at that time [28].

The Statens Serum Institute was until the 1966 the center of microbiology in Denmark, and all the professors of bacteriology at the University of Copenhagen were recruited from the institute until 1989. In 1965, the new medical speciality 'Clinical Microbiology' was approved by the Board of Health and Statens Seruminstitut, therefore, gradually lost its diagnostic importance in Denmark during the following 25 years [28].

Christian Gram

Hans Christian Joachim Gram (called Christian Gram, 1853–1938) initially studied natural science (botany) at the University of Copenhagen [31,32] and assisted professor of zoology J. Steenstrup (1813–1897) who convinced Gram to study medicine, but Gram also continued to work with botany at professor Eugen Warming's laboratory who had published the first Danish bacteriological article in 1876 [21]. During his medical study Gram and his fellow students admired the novel bacteriological work of Salomonsen, who at that time worked as a young medical doctor at Kommunehospitalet in Copenhagen. The knowledge of botany, microscopy and natural science was of great advantage and decisive for Gram's career. He changed to study medicine and graduated as a medical doctor in 1878. He received a gold medal from the university for a scientific paper and continued his research work which was accepted as a doctoral thesis in 1883 'Investigations of the size of erythrocytes in humans'. When Salomonsen organized his first laboratory course of bacteriological techniques in the spring (3 months) and autumn (2 months) in 1883 Gram was one of the 11 participants [24]. Gram had planned to visit famous bacteriologists in other countries after finishing the course and Salomonsen gave him a letter which introduced him to the pathologist Carl Friedländer at Friedrichshain Hospital in Berlin. Salomonsen knew Friedländer from his own visit to Koch in 1882. Gram arrived at Friedländer's laboratory on October 22, 1883, and worked there until March 20, 1884 [31]. Several staining methods were available for bacteriology at that time, but when they were applied on tissue from infected organs both bacteria and nuclei from the cells of the patients or the experimental animals were stained. It was therefore difficult to distinguish bacteria from nuclei in the tissue cells. Friedländer studied the ethiology of pneumonia and he had detected capsulate bacteria in autopsies from patients who had died of pneumonia [31]. Gram had already worked on development of a combined staining method for sliced samples from kidneys. He employed aniline-gentiana violet and iodine solution to obtain blue nuclei and brown urinary cylinders. The aniline-gentiana violet stained samples were usually difficult to decolorize by ethanol but when he added iodine solution after the aniline-gentiana violet staining the samples became completely decolorized by ethanol. This observation was the background for Gram's development of his famous staining method by which pneumococci, the common causative agent of pneumonia, were more intensively stained than by any of the other staining methods whereas nuclei and other tissue components were completely decolorized by ethanol. It was in fact the first method which stained bacteria but not nuclei [31,33]. The time used for Gram's staining method was 15 min. For ordinary bacteria but 12–24 h for mycobacteria. Gram employed staining with Ehrlich's aniline-gentiana violet (or fuchsin) followed by Lugol's iodine solution in water (as mordant), which at that time was frequently used by bacteriologists for staining of specimens. Gram observed that a number of different bacteria were stained black-reddish whereas other *e.g*. typhoid bacteria were decolorized, but that they (and the tissue components) could be visualized by counterstaining with *e.g*. Bismarck brown or vesuvin [33]. Interestingly, Gram's staining method has only been slightly modified since 1883. The current modification employs crystal violet followed by iodine solution in water, decolorization with ethanol and counterstaining with fuchsin and the procedure can be completed in 3 min. The significance of Gram's staining method became immediately clear to him since he observed severe problems in Friedländer's interpretation of the ethiology of pneumonia because he mixed up the pneumococci observed by microscopy with the *Klebsiella pneumoniae* he cultured from the patient and used for animal experiments [31]. There is little doubt that Gram realized the general importance of his staining method and so did the scientific community because it divided bacteria in two large unrelated groups, Gram-positive and Gram-negative. This has crucial implications not only for the identification of bacteria and taxonomy but also as rapid diagnostic tool in the daily work in bacteriological laboratories all over the world. It took, however, many years until the biochemical and structural background for the Gram-differential staining method was explained by the difference between the cell walls of Gram-negative and Gram-positive bacteria [34]. Gram, however, changed his career to pharmacology where he became professor (1891–1900) and later at the Department of Internal Medicine A at Rigshospitalet, where he also became professor and physician-in-chief (1893–1924) until he retired [32].

THE DEVELOPMENT OF VETERINARY MICROBIOLOGY IN DENMARK

Bernhard bang

Bernhard Bang (1848–1932) first studied medicine and completed his medical studies in 1872, one year later than Salomonsen who became his friend [35]. Bang had got a strong interest in research and thought he might be able to get into touch with such work by studying animal anatomy and animal diseases, and already after less that 2 years he also passed the veterinary examination in 1873. He practiced as a physician and became prosector (1874) and later assistant physician (1877) at the Kommunehospitalet in Copenhagen at the time where Salomonsen was also working in the hospital (1873–74) and did his first bacteriological diagnosis as described above which Bang may have heard about. During his medical education Bang also became well acquainted with antiseptics and histological investigations. In 1880, Bang received an offer to get a position at the Veterinary College in Copenhagen and after having visited veterinary colleges in Berlin, Dredsen, Vienna, Munich, Stuttgart and Paris, he accepted the position [35]. With his background he may be regarded as the first 'One Health' scientist combining human medicine and veterinary medicine. Bang soon began to devote himself to bacteriology and was trained by his friend Salomonsen and participated in Salomonsen's first laboratory course of bacteriology in 1883 [24]. Bang became professor in 1892. Bang's scientific work can be divided into three areas. The first area comprises his investigations on many important animal diseases (*e.g*. actinomycosis, mastitis in cattle, bacteria causing necrotic infections, endocarditis in swine, epizootic abortions infections, chronic bacterial enteritis in cattle (Johne's disease) and abortion due to tuberculosis) [35]. The second area comprises Bang's investigations on tuberculosis of the udder and tuberculous milk [35]. The third area comprises Bang's work on tuberculin and its application for diagnosing and combatting tuberculosis in cattle by means of a method developed by himself [35]. Bovine tuberculosis was causing 25% of intestinal and glandular tuberculosis in the Danish population due to the presence of the bovine mycobacteria in the milk [30,35,36]. Bang became world famous and after his retirement got a lifelong annual gift from the Danish state. As he pointed out, he had the good fortune to be plunged into the study of animal diseases at a time when the greatest progress ever seen in the history of medicine was just taking place.

THE DEVELOPMENT OF TECHNICAL MICROBIOLOGY IN DENMARK

Emil Chr. Hansen

The technical/industrial branch of microbiology was pioneered by Emil Christian Hansen (1842–1909) who was educated as a house painter but subsequently he studied botany at the University of Copenhagen [37]. In 1876, he received a gold medal from the university for an article about fungi. The Carlsberg brewery was founded by J.C. Jacobsen (1811–1887) in 1847 who believed that insight into chemistry and associated sciences would lead to the best beer in Europe. He therefore founded the Carlsberg Laboratory in 1875 to study the scientific background for brewing beer. The Carlsberg Laboratory had two departments, Department of Chemistry and Department of Physiology, and was closely supervised by Jacobsen. After his death, the Carlsberg Laboratory was supervised by a board of 5 members, three of whom were members of the Royal Danish Society of Science and Letters elected therefrom to the Carlsberg Foundation (established in 1876). The inspiration came from Pasteur's (1876) 'Ètudes sur la biére. Ses maladies, causes qui les provoquent, procédé pour la rendre inalterable, avec une theorie nouvelle de la fermentation' [7] and Jacobsen had bought a sculpture of Pasteur made by the French sculptor and painter Paul Dubois (1829–1905) which he placed in the laboratory. Hansen began to work half-time in the Carlsberg Laboratory in 1877 and half-time in the laboratory of professor of physiology Peter Ludvig Panum (1820–1885), the founder of experimental physiology in Denmark. Hansen defended his doctoral thesis 1879 'Organisms in beer and beer wort' and became the head of the department of physiology at the Carlsberg Laboratory the same year.

The results of Pasteur's studies of the diseases of beer could not always prevent that production of beer sometimes resulted in a bad tasting and smelling product and economic losses and that happened several times in 1880–82 at the Tuborg brewery in Copenhagen and in 1883 at the Carlsberg brewery. Hansen studied the sediment of the beer fermentation with his microscope and detected that it contained 'wild' yeast in addition to the brewery's own *Saccharomyces yeast* [37–41]. Hansen succeeded in production of a pure yeast culture, which he named *S. carlsbergensis* and that strain was therefore used for production of beer ever since. This important result became known in other countries and a few years later pure yeast strains became the normal procedure for beer production in large breweries all over the world. Hansen started his work on yeast fermentation in 1879, and in the period 1881–1908, he published 13 articles under the headline 'Investigations of alcohol producing yeast's physiology and morphology' [38–41]. Some of his work was published as an English book in 1896 [42]. Furthermore, together with collaborators on the Carlsberg brewery Hansen designed and produced a yeast propagation equipment for growing *S. cereviciae* under controlled conditions and that paved the road for large scale beer production. The propagation apparatus was used for the next 100 years. Hansen was an exceptionally gifted laboratory scientist who invented and adapted new methods for his studies and got inspiration from visits to both Koch's, Max Delbrück's (1850–1919) and Pasteur's laboratories.

Pasteur was the star of the International Medical Congress in Copenhagen (August 1884) where he gave a lecture about pathogenic microbes and vaccines, including his new results of his rabies research [8]. He was the personal guest of Jacobsen in Hotel d'Angleterre and visited the Carlsberg brewery and the Carlsberg Laboratory where he discussed Hansen's new results [37]. Hansen visited Pasteur in Paris 1885 and was invited to his home and Pasteur promoted that Hansen received the Gold Medal from La Societé d'Encouragement pour l'Industrie nationale 1886 and many more awards of honor followed [37]. There are some personal notes from Hansen about his visit to Pasteur in Paris and Pasteur's visit to him in Copenhagen [37]:

> Hansen: 'Pasteur – the Pope in Paris'.Hansen: 'It has been remarkable for me to see how common it is in Paris to see women with quite a strong development of

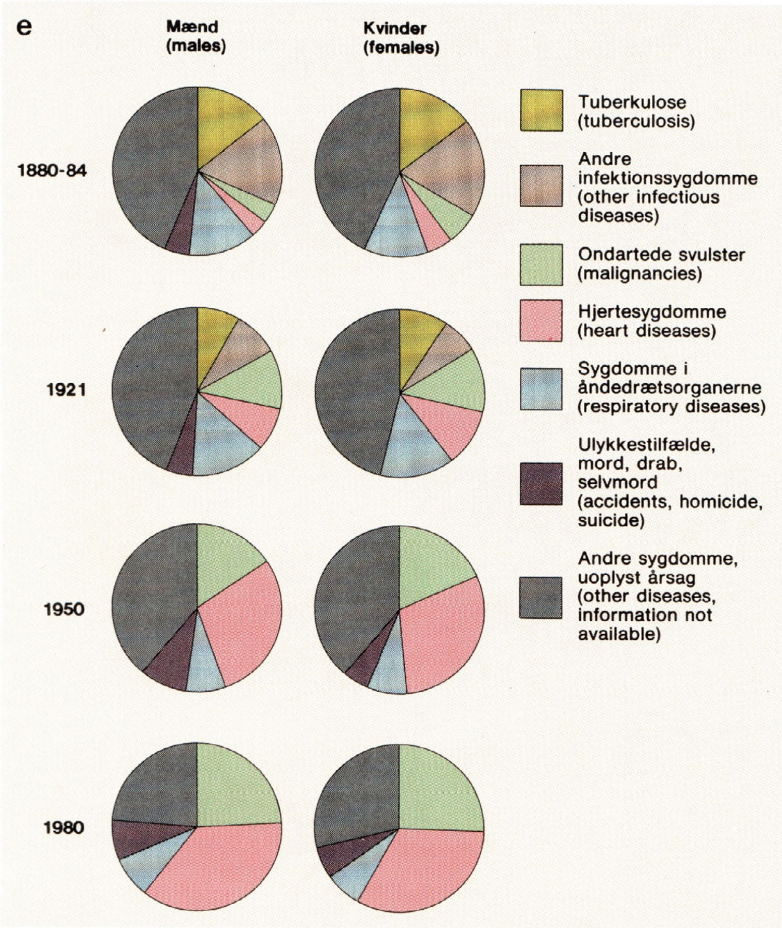

Fig. 2. Causes of death (%) for men and women in Denmark 1880–1980 [43].

beard'.Pasteur: 'The Danes are a quiet, serious people who do not understand jokes and always suffer from headache'.Hansen about Koch after a visit to Koch's Gesundheitsamt in Berlin 1882 [37]:

Hansen: 'Nobody can match him neither in skillfulness nor in arrogance'.
Hansen: 'The close connection to the state administration, public advertisement and ruthless self promotion without any decent limits is a consequence of the changing times and strong competition'.Emil Chr. Hansen became world famous and his methods were used all over the world and he was called 'the Pasteur of the Nordic countries'. He was the founder of Danish biotechnology and technical microbiology which was and is used in the brewing-, the pharmaceutical- and the enzyme industry [37].

CONCLUDING REMARKS

The greatest progress in human and veterinarian medicine and in other fields, such as the brewing-, the pharmaceutical- and the enzyme industry, was initiated by Louis Pasteur. In Denmark, like in other countries, the new and revolutionary microbial field of research attracted the very best young students many of whom later became world famous scientists. In Denmark, the pioneers Carl Julius Salomonsen, Christian Gram and Thorvald Madsen were medical doctors and in the veterinary field Bernhard Bang was also a medical doctor who subsequently also graduated as a veterinary doctor. In the technical/industrial field, Emil Chr. Hansen was educated as a botanist with special interest of fungi. These pioneers and their students and assistants completely changed human and veterinary medicine, which led to scientifically based prevention and treatment of infectious diseases first using *non-pharmacological interventions* and with the advance of vaccines and antibiotics also *pharmacological interventions*, which we generally are still using during the current Covid-19 pandemic. In Denmark, more than half of the known causes of death 1880–84 was infectious diseases and this changed completely the following decades where cancer and cardiovascular diseases became the predominant causes of death (Fig. 2). In the technical/industrial field, a similar revolution took place in Denmark involving, *e.g*., breweries, food production, enzyme production and pharmaceutical industry, and thereby contributing to pave the way for a much more wealthy society which could afford the development of our welfare state. These pioneers followed in their scientific work the famous saying of the old roman poet Horats '*Carpe Diem*' inspired by the work of Louis Pasteur.

CONFLICT OF INTERESTS

I have no conflict of interests.

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