Panum's studies on "putrid poison"1856

An early description of endotoxin

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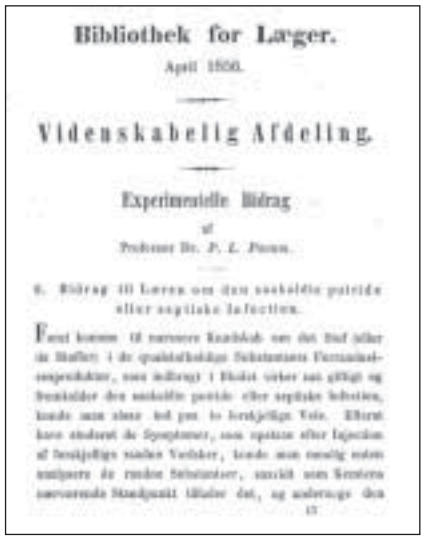
Dan Med Bull 2006;53:450-2

ABSTRACT

In 1855-1856 the Danish physiologist, Peter Ludvig Panum (1820-1885) performed a series of remarkable experiments on "putrid poison", a hypothetical substance claimed to be responsible for the symptoms and signs seen in patients with sepsis. Dogs were given intravenous infusions of putrefying solutions, and symptoms and signs were recorded. Infusion of a suitable amount resulted in characteristic sepsis symptoms and signs, which only started after a delay of half an hour. By modifying his test solutions Panum could show that the toxic principle was a solid substance, soluble in water, but insoluble in alcohol, and with preserved activity after long-term boiling. "Putrid poison" has striking similarities with endotoxin, a cell wall product of Gram-negative bacteria and a powerful inducer of inflammation and septic shock. Thanks to Panum's carefully arranged experiments and meticulous recording of observations it is fair to conclude that "putrid poison" was endotoxin, and as such he deserves credit for being the first to have described endotoxin. Panum published his observations twice, in Danish in 1856, and in German in 1874. At first he rejected the possibility that bacteria could play a causative role in the development of symptoms and signs seen after infusion of "putrid poison". However, in his last publication he hypothesized that "putrid poison" could be a bacterial product, and he envisaged future antibacterial chemotherapy of sepsis and treatment with anti-endotoxin agents.

The Danish physiologist, Peter Ludvig Panum (1820-1885) (Figure 1) made several important contributions to the study of infectious diseases. He gained international reputation for his epidemiological studies of measles, however, his work on "putrid poison" is equally important, though much less famous (1-3). "Putrid poison" has striking similarities with endotoxin, a cell wall product of Gram-negative bacteria and powerful inducer of inflammation and septic shock (4, 5). This year marks the 150-year anniversary of his experiments, which were published in Danish in April 1856 (Figure 2) (2), and re-published in German 18 years later (3). It has previously been suggested that Panum should be credited with the discovery of endotoxin, however, this has not gained full international recognition (5, 6). In this paper his two publications are revisited in order to evaluate in further detail, whether "putrid poison" was endotoxin or not.

Figure 1. Peter Ludvig Panum (1820-1885), 1867. Courtesy: Medical Museion, University of Copenhagen.



Bibliothek for Læger.

April 1856.

Videnskabelig Afdeling.

Experimentelle Bidrag

af

Professor Dr. P. L. Panum.

Figure 2. Front page of Panum's publication on putrid poison in the Danish medical journal, Bibliotek for Læger, April 1856 (2).

EXPERIMENTS WITH "PUTRID POISON"

Panum performed his studies in the winter 1855-1856, while serving as professor of pathology and physiology at the University of Kiel in Holstein (7-9). Animal experiments on the pathogenesis of embolism prompted him to study septic embolism and pyaemia. At his time a hypothetical substance, called "putrid poison", was held responsible for the symptoms and signs observed in patients with sepsis. In the search for the nature of this substance, he performed a series of experiments with dogs. They were given intravenous infusions of putrefying solutions, produced from dog's meat in distilled water, which had been left to decompose at room temperature, until it was strongly malodorous. Before infusion the solutions were filtered through a series of paper filters of decreasing pore size, ending up with a product that was macroscopically clear and free of particles and bacteria at microscopy.

Panum observed that infusion of a large volume resulted in immediate collapse and death of the dogs. However, if they received a minor volume (24-36 ml), symptoms only occurred after an incubation time of about ½ hour. A characteristic pathological picture then developed, starting with malaise, rigors and vomiting, followed by prolonged tenesmus and defecation. The dogs bristled, pupils became dilated, and the conjunctivae red injected. Gradually this led into a picture of vascular collapse, which either resulted in death within a few hours, or slow restitution over the next days. Autopsy of fatal cases typically showed hyperaemia and bleedings of the gastric and intestinal mucosa, whereas the other organs appeared normal. Blood from the heart often contained many small dark coagula dispersed in non-coagulated blood.

Panum's next step was to modify the basic test solution and examine the reactivity of each modification in the same experimental setup. He made nine experiments, as outlined below (2, 3).

1. A dog was given 24 ml of non-modified stock solution prepared as described above. It reacted with typical symptoms and signs and died after six hours. Autopsy findings were as described above.
2. A distillate of the stock solution was given to a new dog, which reacted with no symptoms.
3. A filtrate of the remaining fluid in the retort, which had been heated to 90-100°C for 11 hours, was given to the dog used in

experiment 2. It elicited the same complex of symptoms as seen in experiment 1. The dog survived and recovered over the next nine days.

4. A major volume of the stock solution was evaporated to dryness, and the residue was extracted with absolute alcohol. The alcoholic extract was evaporated to dryness, re-suspended in distilled water, and infused into the dog used in experiments 2 and 3. The dog became drowsy, but showed no signs of toxicity.
5. This experiment was a repetition of experiment 3, but with a new dog, which reacted with typical symptoms and signs. It survived and recovered over the next days.
6. This experiment was a repetition of experiment 2, using the same dog as in experiment 5. It reacted with no signs of toxicity.
7. Panum had observed that an albumen-like substance was precipitated upon prolonged boiling of the stock solution. In order to test if this substance was toxic he performed the following experiment: After filtration, the residue on the filter was triturated, re-suspended in distilled water, and infused into a new dog. It reacted with typical symptoms and signs, and recovered gradually over the next eight days.
8. In order to test if toxicity was due to the albumen-like substance itself or to soluble toxins adsorbed to its surface he extracted the filtrated residue first with absolute alcohol, then with cold, and finally with boiling water. The residue was then treated in the same manner as in experiment 7 and infused into the surviving dog used in experiment 7. This time the dog showed no signs of toxicity.
9. Finally, a major volume of the original test solution was evaporated to dryness, and the residue was extracted, first with absolute alcohol, and thereafter with cold and boiling distilled water. The watery extract was filtered and infused into a new dog, which reacted with typical symptoms and signs. It survived the first time, but was sacrificed afterwards. Autopsy findings were as described above.

Based on these experiments Panum concluded that "putrid poison" was a solid, non-volatile substance, which was soluble in water, but not in alcohol. It was not decomposed by prolonged boiling or evaporation even to completely dry residue. The albuminoidal contents of putrefying solutions were not poisonous per se, but "putrid poison" could adsorb to their surface. In its intensity of action it was comparable only to snake venom, curare, and plant alkaloids (2).

POINTS OF RESEMBLANCE BETWEEN "PUTRID POISON" AND ENDOTOXIN

As noted by Panum, his stock solutions contained rod shaped bacteria ("vibrios") before filtration. It can therefore be taken for granted that they also contained endotoxin. "Putrid poison" has several important features in common with endotoxin:

1. Both are solid, water soluble substances, which resist long-term boiling.
2. Intravenous infusions of suitable amounts induce fever and septic symptoms.
3. Symptoms and signs appear after a typical delay of $^1/_2$ hour.
4. Autopsy findings indicate that "putrid poison", like endotoxin, gives rise to organ inflammation and signs of disseminated intravascular coagulation.

Of particular importance is the observed resistance to long-term boiling and the delay in onset of symptoms, which are particularly distinctive features of endotoxin (4). It is also notable that none of the observations in the nine experiments contradict the hypothesis that "putrid poison" could be endotoxin.

Panum reused dogs for several of the experiments (3, 4, 6, 8). This could have biased his "negative" results severely, because endotoxin may induce tolerance (4). By pure luck, he avoided this problem, because the order of experiments implied that the reused dogs had either not been exposed to endotoxin in the foregoing experiment (the dog in experiment 3), or the test solution used in the actual experiment would not contain endotoxin (experiments 4, 6, 8).

For the moment, Panum's work attracted little attention from the international scientific community, presumably because it was published in Danish. In 1859 a short extract of his article was translated into German, apparently without his knowledge (3, 9, 10). This served as an inspiration for other investigators, but did not provide many details. Panum himself planned to publish a more detailed German translation; however, the war between Denmark and Germany in 1864 interrupted his plans, and he was forced to leave Kiel. Later the same year he was appointed professor of physiology at Copenhagen University, and apparently he did not work with "putrid poison" for the next ten years. Others went on with studies of "putrid poison", in part inspired by Panum (3, 9). His results gave rise to some criticism, however, this was mainly due to misunderstandings, which could be attributed to the lack of access to details in his original publication (3). At length several of his observations were confirmed by others. Thus Hemmer (1866) in his studies confirmed that "putrid poison" is insoluble in alcohol, but soluble in water and resistant to boiling (10). Rosenberger (1882) also confirmed the heat stability of "putrid poison" (10).

CONNECTION OF "PUTRID POISON" TO BACTERIA

The nature of "putrid poison" puzzled Panum's mind. He was faced with two possibilities, which both seemed improbable: It could be a chemical substance, or it could be microorganisms, in particular vibrios, which he had frequently detected in the putrefying fluids. In his first publication he rejected both possibilities, the former because of the characteristic delay in onset of symptoms, which was inconsistent with any known chemical substance, the latter because toxicity was not abolished by prolonged boiling. So he left the question open (2).

In 1856 the clinical role of bacteria was very poorly understood, and the miasma theory was still the prevailing explanatory model of infectious diseases. Over the next two decades the germ theory came into evidence, primarily supported by Pasteur's works on fermentation and Lister's work on the effect of antiseptic treatment of wounds (11). In the early 1870s several investigators demonstrated streptococci in pus from infected patients and claimed that they played a causative role in disease. Among them was the young Danish bacteriologist Carl Julius Salomonsen, who performed his first clinical studies on pyaemia in Copenhagen at the end of 1873 (12). Although bacteria had come in focus, their role in disease was still a very controversial issue. This prompted Panum to reconsider his experiments, and in 1874 he re-published his observations on "putrid poison" in Virchow's Archive (3). Compared with his first publication, he had modified his views considerably in favour of a microbial origin of "putrid poison", although he was still sceptical and warned against any hasty conclusions. Remarkably, he no longer considered a chemical or microbial origin as mutually exclusive in explaining the nature of "putrid poison". In his own words: "Maybe this poison is produced through the life process of bacteria, or to be more explicit probably through the small rods named Bacterium termo *Cohn*, and it seems to be produced in a way analogous to ergotine" (author's translation) (3). In the time of Panum Bacterium termo was a designation of small rod-shaped organisms ("vibrios"). We cannot say with certainty that they were Gram-negative (Gram staining was only invented in 1884 (11)), but in all probability they were. So Panum came very close to the truth about the nature of "putrid poison", although he seems to have regarded it a secretory product (i.e. an exotoxin) rather than an integral part of the bacterial cell.

CLINICAL ASPECTS

Panum had a remarkably clear idea about the clinical significance of

his discoveries. In principle, he envisaged both antimicrobial chemotherapy and anti-endotoxin therapies of sepsis, as may be seen from the following paragraphs of his 1874 paper (author's translation): "If it would turn out through investigations … that ‚putrid poison' is only mediated by Cohn's Bacterium termo … this would be welcomed as a great step forward … Instead of searching for drugs to neutralize ‚putrid poison' … the main indication would be directed against the bacteria, trying above all to kill them, or even better to keep them away. If one succeeded with that ... putrid poison would not be produced … In a practical context putrid poison would keep some significance, since in spite of all prophylactic measures there would still be cases where its production cannot be prevented, and consequently one would have to combat not only the bacteria, but also directly their potential product ‚putrid poison'." (3).

Panum's introduced his ideas decades before Ehrlich, Fleming and Domagk, through their ground-breaking discoveries in the first half of the 20th century, made antimicrobial chemotherapy a clinical reality (11). During the 1990s anti-endotoxin antibodies were tested as a supplement to antibiotics in the treatment of septicaemia, but results were equivocal and on the whole disappointing (13). Nevertheless the basic idea of immunomodulatory therapy is still clinically relevant, and at length this part of Panum's vision may also come true.

Contrasting with the situation in 1856, Panum's 1874 publication immediately aroused great scientific attention, and Pasteur had it translated into French (9,10). In addition to his considerations on the management of septicaemia, the article also contains a footnote in which Panum hypothesized that leukocytes might play a role in killing bacteria, which had invaded the bloodstream (3). Élie Metchnikoff (1845-1916) later stated that this footnote inspired him to the discovery of phagocytosis, for which he won the Nobel Prize in 1908 (9-11).

PANUM'S LATER CAREER

When Panum took up the appointment as professor of physiology at the University of Copenhagen, he insisted on having built a new physiological institute. It was inaugurated in 1867, and provided excellent facilities for experimental laboratory medicine. He became the tutor of a whole generation of brilliant young scientists, and thereby had an enormous impact on the development of laboratory sciences in Denmark. Among his pupils were the physiologist Christian Bohr (1855-1911), the zymologist Emil Christian Hansen (1842-1909), and the medical microbiologist Carl Julius Salomonsen (1847-1924) (9,12).

CONCLUSION

Thanks to Panum's carefully arranged experiments and his very detailed description of test results it is fair to conclude that "putrid poison" was indeed endotoxin. Panum therefore deserves full credit for being the first to have described the biological effects of endotoxin. He also realized that it could be a bacterial product, and he envisaged future therapy of sepsis with antibiotics and anti-endotoxin drugs. His two publications on "putrid poison" are of outstanding quality and should be regarded as key documents in the newer history of infectious diseases (2, 3).

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