

The history of blood cultures: From the research laboratory to the bedside

“In order to study the characters of any species of bacterium it is necessary to have it growing apart from every other species. . . . When we have succeeded in separating it, and have got it to grow on a medium which suits it, we are said to have obtained a pure culture.”

Dr. Robert Muir, pathologist, *Manual of Bacteriology*, 1897¹

THE CASE OF ENDOCARDITIS presented in this issue of *Cleveland Clinic Journal of Medicine* highlights the heterogeneity of the cutaneous manifestations of this disease, as well as the importance of blood cultures in making the diagnosis.² A patient develops a fever, blood cultures are done, and *Staphylococcus aureus* grows. Next step is to check an echocardiogram to find the source of the bacteremia and, lo and behold, vegetations are found and the boxes of the Duke criteria for endocarditis are checked (2 major criteria). The patient had multiple rashes consistent with endocarditis, but what cemented the diagnosis was the blood culture leading to the echocardiography findings.

Associate Editor, Adam Brown, MD, discusses an angle related to the article “Skin manifestations in a patient with acute bacterial infective endocarditis” on page 657.

We consider blood cultures to be an essential component of an infectious disease workup, especially in a patient in whom bacterial endocarditis is suspected. It's reasonable to think culturing of blood was adopted rapidly in clinical practice around the time of the microbiology revolution led by Koch, Pasteur, and Lister, but culturing of bacterial organisms was initially a complex and labor-intensive process relegated to the research laboratories across the United States and Europe. It

wasn't until endocarditis became a recognized clinical entity in 1885 and the hunt began in earnest to prove the etiology was bacterial that blood cultures were brought to the bedside.

■ FROM COMPLEX BEGINNINGS . . .

The *Manual of Bacteriology*, first published in 1897, is a just over 500-page textbook of the knowledge at the time of the rapidly expanding field of microbiology.¹ The textbook walks the reader through the multiple processes for culturing and isolating bacterial organisms, starting with sterilizing of equipment: dry heat in a hot air chamber, wet heat in Koch's steam sterilizer, or a high-pressure steam chamber. Next, the book outlines multiple practices for culturing bacteria with an amalgamation of recipes ranging from ox meat, horse meat, gelatin, agar, blood agar, potatoes, and bread paste.

It took decades of trial and error to develop recipes to create ideal culture media to isolate and grow various organisms. Raw meat was the most popular culture medium, which isn't surprising as bacteria that infect human tissues were the most studied. Many of the bacteria that infect human tissue are also capable of colonizing horse and ox meat. Meat culture had a few negatives, however. For one, the preparation was complex and time consuming.

“It ought to be from an animal recently killed, and should therefore be markedly acid to litmus paper. It must be freed from fat, and finely minced. For each pound of mince add 1000 cc distilled water, and mix thoroughly in a shallow dish. Skim off any fat present, removing the last traces by stoking the surface of the fluid with pieces of filter paper. Set aside in a cool place for twenty-four hours. Place a clean linen cloth over the mouth of a large filter funnel,

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and strain the fluid through it into a flask. Pour the minced meat into the cloth, and gathering [sic] up the edges of the cloth in the left hand, squeeze out the juice still held back in the contained meat. Finish this expression by putting the cloth and its contents into a meat press . . . squeeze out the last drops.”¹

Even when prepared correctly, the meat-based culture media presented challenges when used to culture bacteria, as, not surprisingly, meat is opaque and colonies of bacteria could not be observed *growing within*. An advancement in culture technology was the recognition that gelatin could be sterilized and added to the culture mixture to make it clearer and allow the viewer to see bacterial growth *within* the meat culture.

Gelatin was also popular as an additive because it could be purchased ready-made (Gold label from Paris was mentioned in the textbook as being particularly high quality). Challenges with gelatin were noted, however, as at human body temperature—the optimal temperature for growing organisms that affect humans—gelatin is a liquid, making it unstable and potentially leading to a plate full of soupy minced meat.¹

A substitution for gelatin came from discovering agar’s stability and ability to cultivate bacterial organisms. Although agar now is most associated with the thing you made to grow bacteria in your Biology 101 lab, originally agar had nothing to do with bacteriology. *Agar-agar* is a southeast Asian term for seaweed. In the late 1600s it was noted that seaweed and algae when ground and left to dry in the sun turned into a semi-solid jelly and could be used as a food additive.

Agar began to be used in research laboratories in the late 19th century, when Dr. Walther Hesse, then a researcher working in Dr. Robert Koch’s laboratory, was having difficulty with the gelatin culture he was applying to the inside of a test tube to grow bacteria, as the gelatin persistently melted in the summertime heat. Legend has it his wife Fanny Hesse, who was working as his unpaid laboratory assistant, suggested using the food additive agar as a culture medium because it is stable at higher temperatures.³ Not only was agar solid at a wide range of temperatures, but it was also clear and able to grow various bacteria. Agar has been a staple in research and Biology 101 labs ever since.

■ DIFFERENT MIXTURES FOR DIFFERENT BACTERIA

Not all bacteria, it turns out, are fans of plain, dried-out, pulverized seaweed. Through much trial and error, different additives or formulations of culture media were created to cultivate and isolate certain, more discerning organisms.¹ For example, glycerine broth

could be added to cultivate the famously fastidious *Mycobacterium tuberculosis*, whereas glucose could be added for diphtheriae. Pfeiffer influenza bacillus (later recognized to not cause influenza) had a predilection for human or ox blood added to agar plates, inspiring its future name *Haemophilus* (heme-loving) *influenzae*. Even bacteria that had a deep disdain for oxygen could be grown by combining sulfuric acid with pure zinc to create hydrogen, which is then passed over the culture to bind and expel the oxygen and make a comfy anerobic environment for certain organisms.¹ Decades of work, trial, and error led to an assortment of culture media to isolate and grow bacteria in the research laboratory.

■ BRINGING BLOOD CULTURE TO THE BEDSIDE IN THE PURSUIT OF ENDOCARDITIS

For centuries endocarditis was an enigmatic disease. It is debatable when the first description of endocarditis occurred. Dr. Jean-Nicolas Corvisart in the late 1700s was the first to use the term *vegetation* to describe a lesion on the mitral valve of a patient who died, but there was no clear overarching disease known to cause these valvular changes.⁴ Corvisart surmised that the vegetations were caused by syphilis.

Other medical heavyweights had hypotheses about the cause of the vegetations. None other than Dr. René-Théophile Hyacinthe Laënnec, the inventor of the stethoscope, hypothesized that vegetations were caused by thrombus formation.⁴

The “clinical entity” *endocarditis* made its debut on the international stage in 1885 when Dr. William Osler reviewed more than 200 cases of the disease in a Gulstonian lecture series in London.⁵ Osler synthesized the data, describing signs and symptoms to look for like fever, joint pain, rash, and splenomegaly. Osler also made the critical observation that a history of valvular abnormalities, such as those resulting from rheumatic fever, predisposes to the development of endocarditis.^{4,6} What was the cause? Osler hypothesized it was infectious but couldn’t prove it. It would take another 3 decades to prove the infectious etiology of endocarditis.

It wasn’t until 1910 that Dr. Hugo Schottmüller cultured viridans streptococci from a patient with endocarditis.^{4,7} That same year, Dr. Emanuel Libman, practicing at Mount Sinai in New York City, published a paper with the confident title “The etiology of subacute infective endocarditis,” along with Herbert Louis Celler.⁸ Libman described 43 patients who died of endocarditis. Blood cultures were done in 36 of these

patients, and “atypical” nonhemolytic streptococci grew in 35.⁴

Libman also reviewed more than 3,000 blood cultures over the preceding 10 years during his studies on the “bacteriology of the blood,” recognizing other causes of endocarditis such as *Staphylococcus*.⁶ He was particularly inclined to make this discovery, as he had previously worked under the mentorship of Dr. Theodor Escherich in Vienna, a famous pediatrician who first isolated a bacterium from the intestines of multiple children he termed *Bacterium coli commune* and who would later have his name attached to the ever-difficult-to spell *Escherichia coli*. Dr. Escherich was particularly known for his skills of bacterial culture and passed these skills to Dr. Libman.⁴

With blood cultures, Dr. Libman showed the bacterial etiology of infectious endocarditis and how, in the right clinical context, the diagnosis of endocarditis could be made in a living, breathing person. Half a century before the development of echocardiography, blood culture gave us 1 of the 2 major Duke criteria to diagnose infectious endocarditis. Before Dr. Libman’s paper, the diagnosis of endocarditis was mostly relegated to the pathologist at autopsy.

CONCLUSION

Culturing and isolating bacteria was a labor-intensive process developed through decades of toil in research

laboratories around the globe. The skills Dr. Emanuel Libman attained working directly with Dr. Escherich allowed him to establish the bacterial cause of endocarditis, paving the way for use of bacterial culture in the clinic to help establish the diagnosis of bacteremia and potentially, endocarditis. Once the antibiotic era opened in the 1940s, there was an even greater desire to diagnose bacteremia, as it was recognized that the rapid introduction of antibiotics could reduce the risk of septic shock and death. Techniques for culturing blood improved, becoming less time intensive, and, thankfully for the horse and ox, less reliant on raw meat. In the 1970s automated growth systems were introduced, detecting evidence of bacterial metabolism and division instead of relying on the naked eye of a human.⁹

Blood cultures have become standard practice for evaluating a patient for suspected infection. Next time you’re on the hospital wards and you’re alerted to fever in a patient with an unknown cause and you go to click the blood culture button, remember the oxen sacrificed, the melted gelatin, and the pursuit of endocarditis that gave us this valuable clinical tool. ■

DISCLOSURES

Dr. Brown has disclosed consulting and teaching and speaking for Amgen and Chemocentryx.

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