

THE INTRODUCTION OF AGAR-AGAR INTO BACTERIOLOGY

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Among fundamental developments in bacteriology the elaboration of solid culture substrates will always hold first place. With the introduction of a method for isolating microorganisms and growing them in pure culture, bacteriologists possessed the means for studying what Ferdinand Cohn (1872) called "Die kleinsten lebenden Wesen." Credit for the discovery of the solid medium technique is commonly accorded to Robert Koch, but, as is usual with discoveries of major importance in science, he had his fore-runners. Of course this detracts in no way from the glory of Koch's achievement; his genius lay in his ability to bring order out of chaos. Starting as it were with a box of miscellaneous beads, varying in size and shape, each bead a scientific fact, he found a thread on which the beads could be strung to form a perfect necklace.

There are, it seems, two types of workers in Science, the bead collectors and the bead stringers. Which of the two is more important must not be a question for discussion here. It is a fact, however, that bead collectors are more numerous than bead stringers and it is also an historical commonplace that the bead collectors are more obscure, less known to the world, than those geniuses who string necklaces. The historian of science must concern himself with both types of workers and their productions. The contributions of the obscure are sometimes as important as those of investigators in a more favorable position to be il-

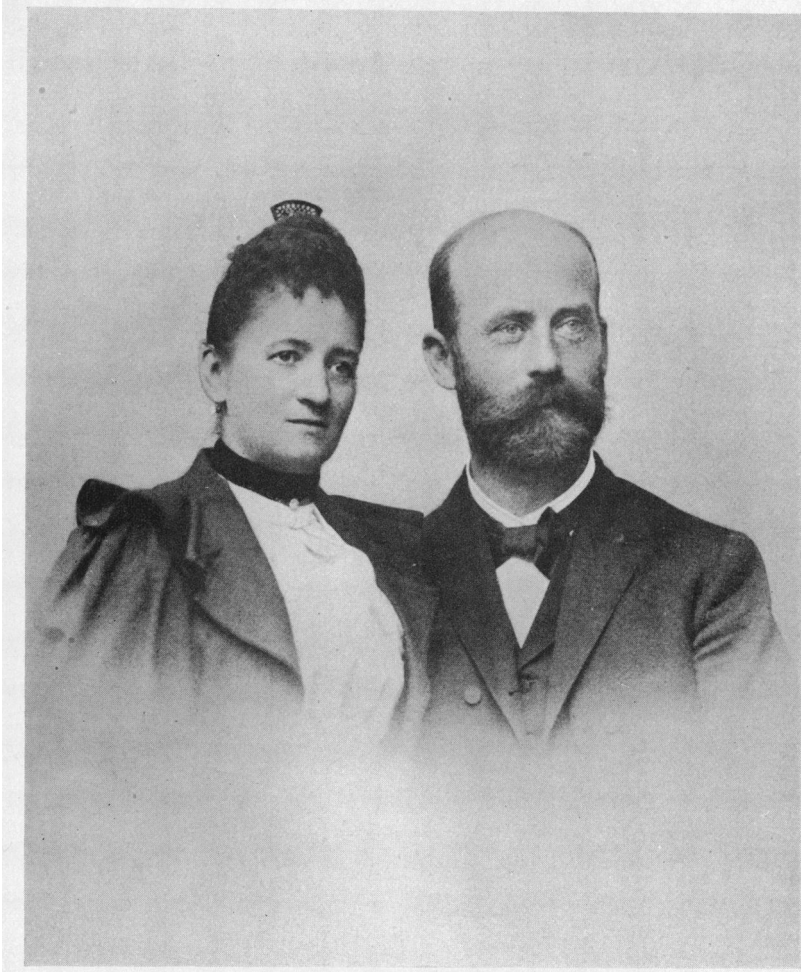
luminated by the spotlight of fame. Yet history does record the work of individuals, who, while generally unknown and unsung have made discoveries of lasting value. As a foreword or "Golden Text" to his volume *Behind the Doctor*, Clendening (1933) quotes from Oliver Wendell Holmes that medicine learned "from a Jesuit how to cure agues, from a friar how to cut for the stone, from a soldier how to treat gout, from a sailor how to keep off scurvy, from a postmaster how to sound the Eustachian tube, from a dairymaid how to prevent smallpox, and from an old market-woman how to catch the itch-insect."

To this list we can now add a housewife and tell the story of how she helped her bacteriologist husband in his studies upon the microorganisms of the air and thereby achieved pure cultures. For many years it has been known to bacteriologists that agar-agar was introduced into bacteriological technique by a certain Frau Hesse. But who Frau Hesse¹ was and how she came upon this important innovation has been until now generally unknown. In August of 1937 we came into the possession of additional facts concerning this discovery. The story of Fannie Eilshemius Hesse is, we feel, of interest to all bacteriologists, but especially to American students of this branch of science. Following the publication of his brief memorial notice in the *Zentr. f. Bakt.*, 1935, we wrote to Professor Edgar von Gierke (1935) asking if he would put us on the track of information we had been seeking for many years. He kindly referred our letter to Dr. Friedrich Hesse, a surgeon now living in Dresden, Germany. He is the son of Frau Hesse and graciously provided us with the facts upon which our story is based. We have also received from him portraits of his parents, here reproduced for the first time. For these facts and photographs, bacteriologists in general owe a debt of gratitude to Dr. Friedrich Hesse.

Frau Hesse was born Fannie Eilshemius in 1850 in a locality now incorporated in Jersey City in the state of New Jersey.

¹ There are two brothers of Frau Hesse still living in New York City, Mr. Henry and Mr. Louis Eilshemius. The latter is a well known artist, his work being the subject of an illustrated paper published in the October (1937) number of the *Magazine of Art*. He has often abbreviated his name to Elshemus.

At that time this community was known as Laurel Hill. Her father had immigrated here from Germany in 1818. We know little of her early life. In 1874 she went abroad, traveling over



FRAU FANNY EILSHEMIUS AND DR. WALTHER HESSE

Europe and finally coming to Germany. It was there she met Walther Hesse, then a district physician in Schwartzenberg, Saxony. Young Hesse was more than a mere country doctor and health officer. He was aware of the new scientific horizons

appearing in the work of Pasteur, Koch, Ferdinand Cohn and many others, for in the winter of 1881-82 he became a student of the then new science of Bacteriology under Robert Koch in the laboratories of the *Kaiserliche Gesundheitsamt*, in Berlin. Many problems were pressing for solution in those early days and although the most important were those dealing with the bacteria causing infectious disease, the problem of the general distribution of these organisms was not neglected. Hesse (1884) began to study the bacterial content of the air.² These studies were continued at his home in Schwartzenberg after his half-year period of residency in the Imperial Health Office. It was in his home, now also his laboratory, that the momentous discovery was made. To understand the importance of this new technical achievement we must review briefly a few facts regarding the historical development of pure culture methods.

As we now know so well from the work of Dobell (1932) and the translation of Cohen (1937), bacteria had been seen and described by Leeuwenhoek just a little over two hundred years before Hesse began his research. During most of the intervening period observers had been content to study them as they were found in nature. Until the time when Pasteur (1857) began his researches on fermentation few individuals had seemed to be interested in whether bacteria could be grown or not. Except to a few botanists, especially Ferdinand Cohn (1872), the problem of the classification of species and genera was of little moment. Most students agreed with Linnaeus (1763) that bacteria belonged in the class *Chaos*; they were little advanced beyond the early classification made by Otho Fridericus Müller (1773). With the studies of Pasteur (1857), Cohn (1872), and Koch (1881, 1882) a new era began. Pasteur, although no systematist, demonstrated the physiological specificity of bacteria. In his studies on fermentation he showed that each type of the organisms which he called by so many different

² The results of this work were finally published in vol. II (1884) of the Contributions from the Gesundheitsamt. The paper is illustrated by three double page chromolithographs. The original paintings for these were made by Frau Hesse. They are in every way excellent.

names was characterized by and responsible for specific physiological activities. In order to prove his contention that specific organisms could bring about alcoholic, lactic or butyric fermentations he devised simple nutrient fluids of known composition to demonstrate these reactions. By transferring minute quantities of fluid containing bacteria from flask to flask he was able to obtain relatively pure cultures. At least they were pure on the basis of the fermentations brought about. There were various attempts to modify his medium and many workers, among them Klebs (1873), Lister (1878), and Salomonsen (1876), attacked the problem of growing pure cultures, with varying degrees of success. The great stumbling block was that they were all working with fluid culture media and the difficulties were almost insuperable. But another group of workers, especially those interested in fungi, were attacking the problem from a more productive angle. As related so dramatically by Harrison (1924) and echoed by Reid (1936) people had for centuries been terrified and awed by epidemics of "blood spots" miraculously appearing on food stuffs, especially the holy wafers. In 1819 an extensive and persistent epidemic of "bloody bread" broke out in Padua. An official investigation was made and one of the investigators, Bartolomeo Bizio, recognized the fungus nature of the blood-red growth. He isolated it from some corn porridge (polenta) and carried out a series of successful transfers to other farinaceous materials. Bizio (1823) named the organism *Serratia marcescens*: *Serratia*, in honor of Serafino Serrati, who was the first to run a steamboat on the Arno, the species name signifying putrefaction. Bizio is not heard of further but we may date the first attempts to grow bacteria on solid culture media from his experiments. Observations were made concerning the more or less accidental growth of bacteria on various organic solid media in the years that followed, but there were none planned with definite and serious purpose until we come to the work of Schroeter (1872), a pupil of Ferdinand Cohn. The paper that he published in 1872 on the chromogenic bacteria is a landmark. He was able to separate various chromogens from each other by growing them on solid media such as potato, coagu-

lated egg white, starch paste and meat. Schroeter found that on these solid substrates his pigment producers often appeared as isolated spots of color. He then learned that all organisms in a single spot or colony were alike. When he transferred organisms from a single colony to fresh media he was able to grow the same organisms and, by repeated transfer, to continue the process indefinitely. He had there the secret of pure culture study but technical difficulties stood in the way of the universal application of his method. The difficulties were due to the presence of nonchromogenic bacteria, especially when the unpigmented colonies closely resembled the color of the medium. It was only a few years later that Koch (1881) entered the field and his genius promptly solved these disturbing problems. He recognized that one must have a substrate which was at once solid, transparent and sterile. What he sought was a universal culture medium, but he soon recognized the impracticability of this quest. He then turned his attention to finding a suitable jelly that could be incorporated with a nutrient fluid. His most promising material was gelatin which the mycologists, among them Vittadini (1850), had been using, for 30 years. With the aid of this medium and his plating and dilution method, Koch (1881) revolutionized bacteriological technique. Isolating pure cultures was, in comparison with the older techniques, enormously simplified. However, the way was not yet completely cleared. Gelatin was not quite the ideal jellifying medium required. It possessed two undesirable qualities. First, it was liquefied by certain organisms. This characteristic, while of value in the identification of a lytic ferment, ruined the medium for the purposes of isolating pure cultures. The second defect arose from the fact that gelatin is not solid but fluid at 37°C. incubator temperature. It was satisfactory for organisms cultivable at room temperatures—22°C., or below—but not for pathogens requiring blood heat.

It was with these difficulties that Walther Hesse and his wife, Fannie, wrestled. Fannie was not merely the "Hausfrau," she was also the technician and the artist illustrator in the home

and laboratory of Dr. Hesse. She cooked not only the soup for her family but also bouillon for her husband's bacteria. In the studies of atmospheric bacteria made by Dr. Hesse (1884), tubes lined with gelatin were used. Measured quantities of air were aspirated through these tubes and from the colonies of bacteria developing on the lining medium the numbers and kinds of microbes in the air were listed. But the maddening liquefaction of gelatin ruined many of the experiments and finally Hesse began to seek new solidifying agents. At this point Frau Hesse became an historic figure. She suggested the use of agar-agar which she had been using for years in her kitchen in the preparation of fruit and vegetable jellies. While yet in America she had received the recipe from her mother; her mother in turn had obtained the formula from some Dutch friends who had formerly lived in Java. In the East Indies, where the source of agar-agar, Japanese seaweed (*Gelidium corneum*), also abounds, this curious material had been used for generations as a jellifying agent and as a thickening for soups (Smith, H. M., 1905).

We can imagine the elation of Dr. Hesse when he set up and studied his tubes lined with his wife's new medium and found that his troubles were at an end. Now he could prepare a substrate, solid, transparent and sterile, which would retain its consistency at all temperatures at which bacteria could grow and which, furthermore, would not be liquified by any of the organisms he encountered in his studies. Without delay the discovery was communicated to Robert Koch by letter, probably late in 1881. Koch recognized its value and made it his own. In 1882, in his now classic preliminary note on the tubercle bacillus, Koch made what is the first printed reference to the use of agar—just one short sentence for a technical improvement so fundamental and epoch making. No formal paper was ever published.

Thus did a modest housewife perform a service to science and to humanity. When she died in 1934, few bacteriologists knew of her death, few perhaps that she had ever lived. Lesser innovations and discoveries are commemorated with the name of

the discoverer. Could not "plain agar" from now on be designated as "Frau Hesse's medium?" Her contribution to bacteriology makes her immortal.

REFERENCES

- BIZIO, BARTOLOMEO. 1823 Lettera di Bartolomeo Bizio al chiarissimo canonico Angelo Bellani sopra il fenomeno della polenta porporina. *Biblioteca Italiana o sia Giornale di Letteratura, Scienze, ed Arti*, Tomo 30, Anno 8, Aprile, Maggio, Giugno, Venezia, 275-295.
- CLENDENING, LOGAN. 1933. *Behind the Doctor*. New York, Knopf.
- COHEN, BARNETT. 1937 *The Leeuwenhoek Letter* (9th of October, 1676). Society of American Bacteriologists, Baltimore.
- COHN, FERDINAND J. 1872 Über Bakterien, die kleinsten lebenden Wesen. *Sammlung gemeinverständlicher wissenschaftlicher Vorträge*, 7, Heft 165, pp. 35. Berlin (also edited for American students by Oswald Seidenstieker, 1889, New York, Holt, pp. 35). Translated in 1881 by Charles S. Dolley with title "On Bacteria, the Smallest of Living Organisms." Reprinted with introduction by Morris C. Leikind in *Bull. Med. Hist.*, 1939, VII, pp. 49-92.
- DOBELL, CLIFFORD. 1932 *Antony van Leeuwenhoek and his "Little Animals."* New York, Harcourt, Brace and Co.
- VON GIERKE, EDGAR. 1935 *Zur Einführung des Agar-agars in die Bakteriologische Technik. Ein Gedenkwort für eine deutsche Ärztin.* *Zentr. f. Bakt.*, Jena, 133, 273.
- HARRISON, F. C. 1924 The "Miraculous" Microorganism. *Trans. Roy. Soc. Canada, Sec. V, Ser. iii*, 18, 1-18.
- HESSE, WALTHER. 1884 Über quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen. *Mitt. aus dem kais. Gesundht.*, Berlin, 2, 182-207.
- KLEBS, E. 1873 Über fractionirte Cultur. *Arch. f. exp. Path. u. Pharmak.*, Leipzig, 1, 31-64.
- KOCH, ROBERT. 1881 *Zur Untersuchungen von pathogenen Organismen.* *Mitt. aus dem kais. Gesundht.*, Berlin, 1, 1-48.
- KOCH, ROBERT. 1882 Die Ätiologie der Tuberkulose. *Berl. klin. Woch.*, 19, 221-230.
- LINNAEUS (KARL VON LINNÉ). 1763 *Genera Morborum in Auditorium Usum.* *Upsaliae.* (p. 32).
- LISTER, JOSEPH. 1878 On the Lactic Fermentation and its Bearings on Pathology. *Trans. Path. Soc. Lond.*, 29, 425-467.
- MERLINO, C. P. 1924 Bartolomeo Bizio's Letter to the most Eminent Priest, Angelo Bellani, Concerning the Phenomenon of the Red-Colored Polenta. *J. Bact.*, 9, 527-543.
- MÜLLER, OTHO FRIDERICUS. 1773 *Verminum Terrestrium et Fluviatilium.* *Haniae et Lipsiae, Heineck et Faber.*
- PASTEUR, LOUIS. 1857 *Mémoire sur la Fermentation Appellée Lactique.* *Compt. Rend. Acad. Sci. Paris*, 45, 913-916.

- REID, ROGER D. 1936 Studies on Bacterial Pigmentation. *J. Bact.*, **31**, 205-210.
- SALMONONSEN, CARL JULIUS. 1876 Zur Isolation differenter bakterien Formen. *Bot. Ztg.*, Leipzig, **34**, 609-624.
- SCHROETER, J. 1872 Über einige durch Bakterien gebildete Pigmente. *Beitr. z. Biol. d. Pflanz.*, Breslau, **1**, 109-126.
- SMITH, H. M. 1905 The Seaweed Industries of Japan. Washington Govt. Ptg. Off. 181 pp.; *Bull. Bureau Fisheries*, 1904, **24**, 133-181.
- VITTADINI, CARLO. 1850 Risultato di Alcuni Esperimenti Istituti sul Baco da Seta e sopra altri Insetti; allo Scopo di Chiarire la Vera Natura del Calcino. *Gior. dell'I. R. Ist. Lombardo di Sci., Let. ed Arti*, **2**, 305-313.