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Escherich and *Escherichia*

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TRIBUTE Herbert C. Friedmann died as this article was being prepared for *EcoSal Plus* (http://www.uchospitals.edu/news/2014/20140117-friedmann.html). Dr. Friedmann was an Associate Professor of Biochemistry and Molecular Biology at the University of Chicago, where he taught biochemistry to undergraduate and graduate students for almost 50 years. Born in Mannheim, Germany, in 1927, Dr. Friedmann and his family fled Nazi Germany after his father was arrested and sent to the Dachau concentration camp. After his father's release, the family moved to India, where he received his BS and MS in chemistry at the University of Madras. He emigrated to the United States and obtained his PhD at the University of Chicago in 1958. After a postdoctoral fellowship at Johns Hopkins University, he returned to the University of Chicago as Assistant Professor of Physiology in 1960. His particular research interest concerned vitamin B_{12} and its role in bacterial nucleotide synthesis. In the second half of his career, he shifted his focus to teaching and writing on the history of biology. He received the highest teaching awards at the University of Chicago and continued to receive glowing reviews from students until his retirement in 2009 at the age of 82. Dr. Friedmann published "Fifty-six laws of good teaching" [http://pubs.acs.org/doi/pdf/10.1021/ed067p413], which included "Always take your students as seriously as they take you" as number 34. In 2006, Dr. Friedmann published a comprehensive biography of Theodor Escherich and his discovery of the organism eventually known as *Escherichia coli* [http://dx.doi.org/10.1016/S0065-2164(06)60005-1]. We are grateful to Elsevier for permitting us to reprint this article in its entirety.

—*James Kaper*

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ABSTRACT The purpose of this essay is threefold: to give an outline of the life and the various achievements of Theodor Escherich, to provide a background to his discovery of what he called *Bacterium coli commune* (now *Escherichia coli*), and to indicate the enormous impact of studies with this organism, long before it became the cornerstone of research in bacteriology and in molecular biology.

> Scarcely two hundred years back can Fame recollect articulately at all; and there she but maunders and mumbles.
>
> (1)

> It is a truth universally acknowledged that there are only two kinds of bacteria. One is *Escherichia coli*, and the other is not.
>
> (2)

THE NAMING OF BACTERIA AND THE "MYSTERY" OF "*E.*"

The names of bacteria provide a double challenge, for, following the footsteps of Carolus Linnaeus for botanical and later for zoological names, one has the convention, convenient and often obscurantist, of giving two names to bacteria. It has been remarked at times that bacterial names are cumbersome. One can say the same of the binomial names of plants and animals, but microbiologists are perhaps more outspoken in their disapprobation:

> Everyone who has worked with bacteria is more or less familiar with such foreign-sounding names as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Phytomonas hyacinthi*, *Thiobacillus thiooxidans*, *Streptococcus lactis*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Proteus vulgaris*, *Clostridium botulinum*, *Actinomyces lavendulae*, etc.
>
> (3, p. 285)

One can add names such as *Shewanella oneidensis, Sulfolobus solfataricus, Moraxella lwoffii, Capnocytophaga ochracea*, and so on. "Based on the whims of discoverer and committees, bacterial names, both general and species, are largely a curious hodge-podge of derivations of the names of scientists, such as *Escherichia coli*—which honors Theodor Escherich—and purely morphological and physiological descriptions, as in *Thiobacillus denitrificans*" (5, p. 163). One of the two names of bacteria is usually more obviously informative than the other, when it refers to disease (*Vibrio cholerae, Mycobacterium tuberculosis*), source (*Leuconostoc mesenteroides*) or an aspect of chemistry (*Methanobacterium thermoautotrophicum, Propionibacterium freudenreichii*). Frequently, one of the two names refers to people, either as the first name, in capitals, such in *Neisseria, Pasteurella, Klebsiella, Ehrlichia, Escherichia, Rickettsia, Yersinia*, or the last name, not in capitals, as in *Pseudomonas stutzeri, Clostridium kluyveri, Bacillus schlegelii, Methanobrevibacter thaueri.*

Such names within names frequently succeed to cloak well-meaning intent in celebratory obscurity. Remembrance fades, and a name remains, glorying in splendid isolation. Matters are particularly nonrevealing when, following convention, the first name is shrunk to the first letter. Thus, while *"E."* may stand for a person such as Ehrlich or Escherich as in *E. equi* or *E. coli*, it may also stand for *Enterobacter* as in *E. aerogenes*, or *Epulopiscium* as in *E. fishelsoni*, or *Eubacterium* as in *E. limosum*. Clarity is retained by the convention of writing out the full name when first encountered in a chapter or an article, but this convention is not always followed. For instance, in newspapers and in Watson's celebrated textbook "The Molecular Biology of the Gene" (e.g., 6, pp. 96 and I-7), one encounters *"E. coli"* like a hieroglyphic, without elaboration or explanation. The present essay is an attempt to rescue the *"E."* in *E. coli* from neglect and obscurity, not as much out of a sense of pedantry, but as a piece of historical remembrance that encapsulates bacteriological insight and bacteriological development.

A PARADOX: THE GOLDEN AGE OF BACTERIOLOGY, AND PERSISTENCE OF NONCONTAGIOUS NOTIONS OF TRANSMISSIBLE DISEASES

The name Escherich (Fig. 1), if remembered at all, is nowadays connected almost exclusively with the name of *Escherichia coli*. It might seem, therefore, that the activities of a well-nigh forgotten physician provide nothing more than a sentimental journey into remote regions of little present-day interest. It will become abundantly clear, however, that the discovery of *Escherichia coli* is the direct result of Theodor Escherich's combination of superb clinician with the recognition that the practice of bacteriology is an intrinsic and necessary part both of clinical medicine and of basic science.

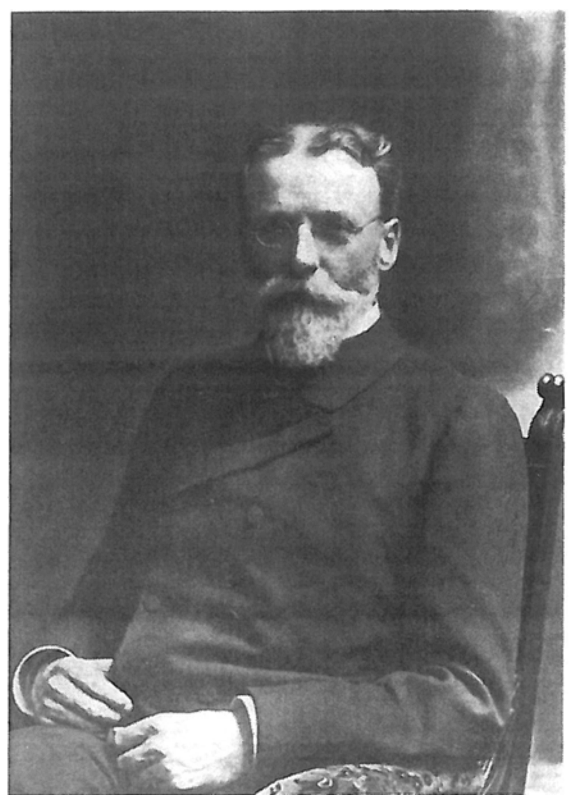


Figure 1 "Escherich was young, distinguished-looking, and wore an impressive beard. This was not unusual at that time" (Béla Schick [4, p. 114] in a moving evocation of his teacher). Copyright 2006, Elsevier, Inc. http://www.sciencedirect.com/science/article/pii/S0065216406600051 doi:10.1128/ecosalplus.ESP-0025-2013.f1

Escherich's discovery in 1885 of the organism eventually named after him falls into the remarkably short and remarkably productive period, 1870s and 1880s, aptly called the golden age of bacteriology (7, 8), which saw the discovery (present-day names) of *Mycobacterium leprae* (1873), *Bacillus anthracis* (1877), *Neisseria gonorrhoeae* (1879), *Salmonella typhi* (1880), *Mycobacterium tuberculosis* (1882), *Corynebacterium diphtheriae* and *Vibrio cholerae* (1883), *Clostridium tetani* (1884), *Streptococcus pneumoniae* (1886), *Neisseria meningitidis* (1887), *Salmonella enteriditis* (1888) (cf. 9, p. 883). Actually it was Filippo Pacini (1812–1883), professor of anatomy at the University of Florence, who already in 1854 discovered the causative agent of cholera and firmly believed that the disease was contagious (5, 10, pp. 289–293; 11, pp. 228, 316). Escherich started his work on digestive diseases in 1884. He was intrigued by the circumstance that *Vibrio cholerae* was at the time the only one of the various pathogenic bacteria known to be directly related to a digestive disease (cf. 12). "It is widely known that the comma bacilli found by Koch in cholera asiatica are the first well-characterized microorganisms cultured from intestinal contents and in turn causing pathogenic activities in the intestinal tract; it can be assumed that all remember their discovery and history" (13, p. 3). He was interested in determining the cause of childhood diarrhea, a devastating disease that caused the deaths of a large percentage of children. Escherich's aim, however, went far beyond an attempt to correlate a given disease with a given microorganism. At the time of his work, it was not generally accepted that diarrhea in children was brought about by bacteria (cf. 14, pp. 80–85). Thus Hermann Widerhofer (1832–1901), the highly respected pediatrician whose chair at the University of Vienna Escherich was to occupy upon Widerhofer's death, maintained a pathological and not a bacterial origin of the disease, and stated this in a section of Gerhardt's Handbook of Pediatrics (15). Widerhofer, incidentally, was the personal physician of the old emperor Franz Joseph I (4, p. 116), an intriguing combination of pediatrics and geriatrics, perhaps in accord with the insight that age is second childishness. These views were in accord with those of Max von Pettenkofer who, as we shall see, at over 70, more or less to cap his influential career, drank a culture of cholera bacteria to prove, once and for all, that the comma bacillus did not cause this disease. The view that diarrhea was mainly chemical and only in part bacteriological was advanced among others by the US physician Charles Delucena Meigs (1792–1869), author of "Observations on Certain of the Diseases of Young Children" (16) and his son John Forsyth Meigs (1818–1882) (with W. Pepper) in "A Practical Treatise on the Diseases of Children" (17). Such ideas, including the notion that foods, especially fats, were the fundamental factors in diarrhea (cf. 18), were subsequently broadened (cf. 19, p. 52; 20). We do not know for certain whether these notions, held in contrast to advancing proofs of bacterial infections, were limited to intestinal diseases, or whether they were thought by some to have wider applications. Even Escherich, early in his career, had his uncertainties. As pointed out by Clemens von Pirquet, his eminent pupil, Escherich, who became known as the father of the school of thought that maintained bacteria to be the cause of diarrhea (cf. 19, pp. 51–52; 4, p. 114) could not in his observations on the 1884 Naples cholera epidemic distance himself from the doubts of his Munich teacher, Pettenkofer, concerning the contagiousness of cholera injections (cf. 21).

THE INVENTION OF THE WORD "BACTERIOLOGY" AND THE RAPID RISE OF BACTERIOLOGY AS A DISTINCT DISCIPLINE

Many of Escherich's late appraisals stressed that he was unusual in being both a microbiologist and a physician. The distinction or division between these disciplines as well as among many others did not, however, exist in his day. Distinctions both divide and enrich. In retrospect, a unified concept and approach is often seen to have achieved more than the specializations imposed on it by the flowering of knowledge. In the words of the eminent historian of science, Frederick L. Holmes (22, p. 74), "Scientific problem areas are more natural than, and often more stable than, the socially constructed disciplines which lay claim to them." I found just one review which recognized that it was not unusual in Escherich's time for physicians to become proficient bacteriologists (23).

The beginning of the word "bacteriology" appears to have been as explosive as the discovery of its objects of attention. The new term pops up independently in 1884 in three locations, the United States, the Continent, and Britain: in March of that year the word is used in a review of an American translation of a French book (24), in August it perceptively emphasizes that a new discipline has arisen, resulting from Koch's discovery of the tuberculosis and cholera bacteria (25), and in November the word refers to a new body of knowledge that has become both obvious and imperative (26).

(i) A review (24, p. 362), March 21, 1884, of George M. Sternberg's translation of Antoine Magnin's "Les Bactéries" (cf. 27) contains the following: "Dr. Sternberg is at the head of the American school of working bacteriologists, if, indeed, he is not its only member." (Sternberg, author of numerous medical treatises, was the principal physician to two US presidents.) (ii) The August 30, 1884, report of the eighth International Medical Congress at Copenhagen (attended by about 700, including Pasteur and Virchow) has: "[...] the discovery of the tubercle bacillus by Professor Koch and his later investigations on the spread of cholera have given such an impulse to this branch of knowledge, that in Germany it has become a separate study under the name of bacteriology" (25, p. 281). (iii) The first sentence of the article "Bacteriology" in the November 20, 1884, issue of *Nature* states: "Among the most striking of the recent rapid advances of science is the development of what we may term bacteriology" (26, p. 49).

The word "bacteriology" rapidly elicited an impressively strong appeal, for within a few years it was found in the titles of textbooks in various countries: in France, 1889, with the first of six editions of Eugène Macé's *Traité Pratique de Bactèriologie* (28); in Germany, 1890, with the first edition of Carl Günther's *Einführung in das Studium der Bakteriologie* (six editions to 1906!) (29) in the United States, 1891, with the first edition of Alexander Crever Abbott's *The Principles of Bacteriology* (cf. 30, pp. 349, 370).

Since *the profession of bacteriologist* did not exist, its initiators had to come from other disciplines, and here the field of medicine predominates by far. The discoverers of the prominent pathogens, such as Hansen, Koch, Albert Neisser, Klebs, Löffler, Babes, and Yersin, were all physicians, and so, of course, was Escherich. Daniel Elmer Salmon was a veterinarian. Eminent early bacteriologists who were not physicians, like Pasteur, trained as a chemist, and Ferdinand Cohn, who started his career as a botanist, did not discover human pathogens.

THEODOR ESCHERICH'S LIFE

From Medical Studies to Assistant in Würzburg

Theodor Escherich was not primarily a microbiologist; rather, he was one of the most eminent pediatricians of his time. His life is easily outlined. He was born in 1857 in the Bavarian town of Ansbach (also the birthplace of Georg Ernst Stahl of phlogiston fame), son of Ferdinand Escherich (1810–1880), a highly respected physician whose many clinical interests included the problem of the high mortality of newborns and ways to improve the care of the poor. These topics, among many others, eventually occupied his son. It has been claimed (cf. 31, p. 303) that the first of Ferdinand Escherich's four wives had been a piano student of Beethoven, but this could not be independently verified. The third wife, Theodor's mother, Maria Sophie Frederike Stromer von Reichenbach, daughter of Johann Sigmund Ludwig Karl Freiherr Stromer von Reichenbach, died when he was 5 years old. Five years later his father moved to Würzburg. At age 12, Theodor, a somewhat rough boy, was sent to the Jesuit seminary Stella Matutina in Feldkirch, northwest Austria. He finished his high school back in Würzburg. His medical studies, 1876–1881, as was usual at the time, were spent in several cities, Strasbourg, Kiel, Berlin, Würzburg. In December 1881, he passed his final medical exam in first class. His career progressed rapidly. From 1882 to 1884, he was an assistant in the medical section of the Julius Hospital in Würzburg. Here he obtained his skills in physical techniques and in diagnostics. The director of this section was Karl Gerhardt (1833–1902), an eminent internist and one of the founders of pediatrics, author of the pioneering "Textbook of Children's Diseases" (1861), and editor of the first German handbook of pediatrics ("Handbuch der Kinderkrankheiten", six volumes and addenda, 1877–1893). Escherich's doctoral dissertation and first publication "Die marantische Sinusthrombose bei Cholera infantum" (32) was written under Gerhardt's sponsorship. In 1883, Escherich published six papers on various clinical subjects, none yet on pediatric topics. Gerhardt was the decisive influence on Theodor Escherich's choice to become a pediatrician. At the time, pediatrics was regarded in Germany as a stepchild of German clinical faculties (cf. 33). Escherich revered Gerhardt throughout his career. Thus, he dedicated to Gerhardt his monographs "Etiology and Pathogenesis of Epidemic Diphtheria" (34) and in his memory "The Tetany of Children" (35). Among the other assistants of Gerhardt at the same time as Escherich was Friedrich von Müller, who became one of the most eminent internists of his time, known among German physicians as Frederick the Great (Friedrich der Grosse). Escherich was promoted to a higher assistantship a year after the start of his employment in Würzburg. A year later, he left to pursue his specialization in pediatrics.

Not everyone respected Gerhardt. In 1885, Gerhardt, an out-and-out clinician, became director of the second internal clinic of the famous Charité Hospital in Berlin. Paul Ehrlich worked in this clinic as a clinician and as a researcher on staining methods. Gerhardt insisted that Ehrlich devote himself to full-time clinical work. This enraged Ehrlich, who developed tuberculosis and left the Charité. He stated later: "When I felt so miserable and forsaken during the time with Gerhardt, I often stood before the cupboard in which my collection of dyes was stored and said to myself: 'These here are my friends, which will not desert me' " (36, p. 28).

Studies in Vienna and Paris: Makings of a Pediatrician

Escherich spent a semester in Vienna to study at the venerable St. Anna Hospital, the oldest German-speaking children's hospital. Here he heard lectures by two of the most eminent pediatricians of the time, Hermann von Widerhofer (1832–1901) and his pupil Alois Monti (1839–1909). He greatly benefited from Widerhofer's ingenious discussions of pediatric cases in clinics (37). Little did he know that he would, in 1902, become Widerhofer's successor. It was in Vienna that Escherich presumably finally decided to remain in the field of pediatrics. Moreover, rather than devoting himself to clinical studies, he began to occupy himself with bacteriological topics. At the Vienna pathological institute, he was apparently the first anywhere to perform bacteriological analyses of mother's milk. He demonstrated that while the milk taken from the first day after birth until 8 months later was sterile, the milk from febrile mothers contained yellow and white staphylococci. As we will see, the broadening of his perspective from pure clinical work was typical for Escherich. This particular pursuit developed into Escherich's later classical studies on the feces of infants. After Vienna, he spent a short time in Paris, where, fluent in French from his time in Strasbourg, he listened to lectures in the Salpêtrière by the world-famous Jean Charcot (1825–1893), who, among others, had also attracted Sigmund Freud. Escherich was also interested in the potential therapeutic application of hypnosis but never used this approach in his own clinics.

Work in Munich: Makings of a Bacteriologist

Following his stay in Paris, Escherich moved in August 1884 to Munich. One reason for his choice was that it was possible to obtain a Habilitation in pediatrics in Munich, although at the time no separate department of pediatrics existed there. He exhibited impressively broad activities: he had access to the hygienic institute of Max von Pettenkofer (1818–1901), the bacteriological laboratory of Otto von Bollinger (1843–1909), the physiological institute of Karl von Voit (1831–1908), and the dairy industry facilities of Franz von Soxhlet (1848–1926). His main interest appears to have been to extend his bacteriological work. He increasingly became convinced that bacteriology could solve or illuminate many pediatric problems. He would sit at the microscope for days and nights on end (38). As luck would have it, he encountered an assistant at the pathological institute, a certain Wilhelm Frobenius, a physician who had learned his bacteriology from the master himself, Robert Koch, during three short visits to Berlin. Escherich came to know Robert Koch's techniques for the cultivation and characterization of bacteria from Frobenius, who in addition gave lectures for physicians interested in these topics. So one could say that Escherich learned his applied bacteriology "straight from the horse's mouth." Escherich's experience with bacteriological techniques led to his work with fecal matters.

As far as could be determined, Frobenius' name is a footnote to bacteriology, since his only mention in the bacteriological world is as Escherich's bacteriology instructor. Apparently, Frobenius published nothing with Koch and nothing later. In 1888, he changed course and became a medical missionary in the East Indies and in the East Asian German possessions (39).

A Study of Cholera in Naples

The year 1884, a year after Koch's discovery of what he called the comma bacillus, later known as *Vibrio cholerae*, was a busy one for Escherich. On the urging of Gerhardt he spent two decisive weeks, October to November, in Naples during an epidemic of cholera in order to study clinical and bacteriological aspects of this intestinal disease (cf. 40, p. 291). It was here that Escherich did his first work on fecal bacteria, published, incredibly, that same year (41). Escherich accompanied the somewhat more senior Rudolf Emmerich from the University of Munich. Robert Koch, in a letter, December 18, 1884, to his friend Carl Flügge, referring to Escherich's publication (1884) of his work with cholera in Naples, remarked that Escherich had no difficulties in finding these organisms, while Emmerich was a miserable failure (cf. 42, p. 159). A fascinating account of the Naples 1884 cholera epidemic is found in Snowden (43).

Carl Flügge (1847–1923) established Germany's first Institute for Hygiene at Goettingen and served as its first director. He became professor of hygiene at the University of Goettingen in 1885, at Breslau in 1887, and eventually at Berlin. In 1886, together with Robert Koch, he began publishing the Zeitschrift für Hygiene, under their joint editorship. The second edition of Flügge's book, "Die Mikroorganismen," published in the same year, already refers to Escherich's 1886 book as a notable beginning of research in this field (ein bedeutsamer Anfang zur Erforschung dieses Gebietes) (44, p. 591, footnote). One is impressed by the thorough reading of contemporary literature, made possible by impressively rapid publications, achieved when typesetting was very much slower than the much more rapid methods used today.

Emmerich was a student of the influential Max von Pettenkofer (cf. 45), Professor of Hygiene in Munich, a man who doubted the bacterial origin of a disease such as cholera, so much so that, at the age of 74, he drank a suspension of cholera bacilli he had asked for from Robert Koch. He developed just a slight bit of dysentery (46, p. 29; cf. 47, p. 183). Koch, guessing Pettenkofer's intent, is held deliberately to have sent him a rather weak suspension of the bacteria. Clearly, therefore, considerations for the life of a fellow scientist outweighed Koch's recognition of acting against the acceptance of his own views on the pathogenicity and infectivity of these and other bacteria. In those days a dispute between the "contagionists" and the traditional "miasmatists," with Koch on the side of the former and Pettenkofer on the side of the latter, had pretty much been settled in favor of the former, and Koch almost certainly recognized that Pettenkofer's views and impending experiments would have only minimal impact. Emmerich and various others, including Elie Metchnikoff, repeated this "experiment" (48). According to Möllers (42, p. 626) and Schlegel (49, p. 162), the culture of *C. vibrio* that Pettenkofer and Emmerich drank was sent not from Berlin by Koch but from Hamburg by Koch's assistant, Georg Gaffky, who had stayed in Hamburg after the 1892 cholera epidemic. Pettenkofer, according to Schlegel, had approached Gaffky to send him a culture of cholera bacteria for research purposes. Research indeed! It is not clear whether Gaffky, or according to some writers, Koch, sent a fully virulent or a weakened culture. While Pettenkofer developed only minor symptoms of cholera, Emmerich almost died after becoming seriously ill. Koch himself, according to this book, regarded such heroic human experiments to be unnecessary, since nature's experiments with cholera epidemics were proof enough.

To Graz, Austria, as Associate Professor of Pediatrics, and Innovations Therein: Spread of Escherich's Fame

Escherich stayed in Munich until 1890 when, at the age of only 33, he was appointed associate (außerordentlicher) professor of pediatrics and director of the St. Anna Children's Hospital at the University of Graz, Austria. He received these appointments not because of his abilities in bacteriology, but because of his achievements as a pediatrician. He rapidly expanded the renown of this hospital to international attention, and more than tripled the number of patients in his hospital. By 1896, the mortality of the neonates in his clinic had been decreased to about 39%, while in the famous Charitè hospital in Berlin it was still as high as 72%. In 1899, a section of the hospital designed for the care of neonates who needed special attention was opened. By 1900, a novel kind of children's walk-in incubator was constructed with his father-in-law, the physicist Leopold von Pfaundler. While in Graz, he studied as many as 300 cases of tetany. He was the first anywhere to use so-called galvanic current as a diagnostic device (cf. 50). In 1895, influenced by von Behring's discovery of diphtheria serum, he wrote his book on diphtheria, croup, and serum therapy (51), and demonstrated that bladder infection was caused by *Bacterium coli commune*. This was not all. A mere 2 years after Roentgen's 1895 discovery of x-rays, Escherich was able to obtain funds for the purchase of an x-ray apparatus that he used to follow bone growth in children after feeding them cod liver oil. With two other physicians he was instrumental in changing the requirements for medical studies, including by 1899 obligatory examinations in pediatrics, dermatology, and psychiatry, reforms that were introduced in Germany only 19 years later (52). Escherich was responsible for expanding research and lecture facilities. He had built a new lecture hall and founded a small library. Four years after his appointment, he was promoted from associate professor (Extraordinarius) to full professor (Ordinarius). His fame spread. He became the pediatrician of nobility and royalty, including the Sultan of Turkey, the son of the King of Bulgaria, and the children of the King of Montenegro. During a journey to Russia, he was called to attend on the hemophiliac son of the czar. Escherich was known, however, not to distinguish between rich and poor patients. As an example, he carried a child suffering from diphtheria from its home to the nearby St. Anna Hospital and in so doing saved the child's life (53; cf. 31, p. 332).

Escherich Attracts Brilliant Students

He attracted a coterie of future eminent pediatricians, including students from the United States. His most famous students were probably Béla Schick and Clemens von Pirquet. Schick is remembered for his intracutaneous test for diphtheria, using the serum that Escherich and Klemensiewicz had developed as early as 1893 (54). von Pirquet coined the word "allergy," introduced a diagnostic test for tuberculosis by the cutaneous reaction to tuberculin (cf. 55, 56), and worked on serum sickness. In 1909, at age 35, von Pirquet spent about a year as the first chairman of the department of pediatrics at Johns Hopkins University (cf. 57). After a short stay at the University of Breslau, he became Escherich's successor, 1911, in Vienna.

To Vienna as Professor of Pediatrics and Director of a Famous Children's Hospital

In 1902, upon the death of Hermann Widerhofer, the eminent pediatrician at the University of Vienna, Escherich was appointed by unanimous decision to succeed him as professor of pediatrics and director of the Vienna St. Anna Children's Hospital. This appointment raised Escherich to one of the most prestigious chairs in pediatrics. [The Vienna St. Anna Children's Hospital was started in 1837. There were only two older pediatric hospitals in Europe, Paris (1802) and St. Petersburg (1834) (cf. 58, p. 1621).]

Various Achievements in Vienna, Medical and Social

Escherich's work in Vienna in the 9 years until his death from a stroke at age 53 was marked by an impressive variety of achievements in many fields of medicine and in the initiation of social organizations devoted to the welfare and health of children. His organizational abilities, already used highly effectively in Graz, again consumed much of his energy in Vienna. He immediately equipped a bacteriology and a chemistry laboratory, and he was the first in Vienna to use x-rays as a diagnostic tool in children. His detailed plan for a completely new children's hospital in Vienna was done by 1906, but bickering with the authorities delayed its completion until after his death. "Because of delay in the building of the new hospital Escherich renovated and expanded the old one ... and created as a first undertaking in Europe, a children's open air terrace on the roof of the new clinic" (59, p. 23). He was devoted to social concerns for the welfare of children and was determined to reduce the capital's high infant mortality. In 1903, just a year after his arrival in Vienna, he appealed for support in a pamphlet to the women of Austria. The response was so strong that in the following year, with imperial patronage and civic approval, he founded the Infants' Care Association (Säuglingsschutz). Previously, newborn babies were not admitted to hospitals because of their high mortality: 20% of all babies died before age 1. His charm and his powers of persuasion induced many high society ladies, including the archduchess, to become members of his organization. Princess Rosa Croy-Sternberg assumed the presidency and each year organized a ball for Vienna's high society that brought in large sums of money for the work of Escherich's baby care society (60, p. 266). A dispensary was started as well as a training school for nurses, who soon became known all over Austria as "Escherich Nurses" (59, p. 23). Public awareness of high infant mortality was very much furthered.

Ernst Weber (1901–1996), born in Vienna, was an eminent engineer with a long career in the United States, recipient of six honorary degrees, member of the National Academy of Sciences, recipient in 1987 of the US National Medal of Science. He was married to Escherich's daughter, Dr. Charlotte Weber (Sonya), born 1895, who had been on the faculty of the School of Medicine at Columbia University. As mentioned in the text, Escherich's only other child, Leo, died of appendicitis at age 9.

Escherich as Vice President and Only European Pediatrician at 1904 Congress of Arts and Sciences, Held as Part of St. Louis World's Fair

A year later, Escherich was invited as the sole European pediatrician to address the International Congress of Arts and Sciences held from September 19 to September 25 at the St. Louis World's Fair. This Congress brought together some of the greatest minds of the time, such as William Osler in Medicine, Jacques Loeb in Biology, Theobald Smith in Pathology and Bacteriology, Adolf Furtwängler (the father of the future conductor Wilhelm Furtwängler) in Archaeology and Classical Greek Art, Ludwig Boltzmann in physics, and a number of past and soon-to-come Nobel laureates in chemistry: Jacobus Hendricus van't Hoff (1901), Svante Arrhenius (1903), William Ramsay (1904), Henri Moissan (1906), and Wilhelm Ostwald (1909). Escherich, representing Austria as one of the seven honorary vice presidents of the

Congress, was one of the speakers at the opening of the Congress. "On the stage were seated the officials of the Congress, the honorary vice presidents from foreign nations, and the officials of the Exposition" (cf. 61, pp. 25, 29–30). Two days later, he gave one of the two addresses in the session on pediatrics (cf. 61, p. 69). This important, long, address, "The Foundations and Aims of Modern Pediatrics," was published five times: in English (62), in German as an excerpt (63), in the complete form (64), in English in the publications of the Congress (65), where he is impressively ennobled as Theodore von Escherich, and some 75 years later again in English (66). A detailed paper (67, 68) reported his American impressions.

Escherich Becomes Famous and Initiates Construction of the Imperial Institute of Maternal and Child Care

Escherich's fame soon spread. In March 1906, he was named Hofrat (court counselor) by the Emperor Franz Joseph. He and his wife were invited several times to dinner at court. The Escherich home, with its soirées and formal dinners for over 40, had become a meeting point of society that included the likes of the composer Gustav Mahler (1860–1911, Escherich's almost exact contemporary) and the opera tenor Leo Slezak (59, p. 27; 31, p. 362). In 1908, Escherich became president of the Austrian Society for Children's Research. On the occasion of the Emperor Franz Joseph's sixtieth jubilee that year, he again drew attention to the inexcusably high national rate of infant mortality, and his efforts eventually led to the construction of the Imperial Institute of Maternal and Child Care.

Bacterium coli commune, a Propellant to Fame

It has to be pointed out that the initial fillip to his ascent, the work that spread his name, was his impressively thorough and intensive study of the intestinal bacteria in neonates and small children. *Bacterium coli commune* provides an important background to his career. "More than a quarter of his publications relate to bacteriology" (69). It has been stated, fittingly, that Escherich transferred Robert Koch's bacteriological methods into pediatrics (70, p. 194). "Although his claims that *B. coli* could cause cystitis and other localized infections were undisputed, his contention that some virulent strains provoked infantile diarrhea and gastroenteritis was verified only after sixty years" (69, p. 404). "*E. coli* is the most common cause of bacterial diarrhea in humans worldwide" (71, p. 617; cf. 72, 73; Sections VI. G and VII). "The idea of the pathogenicity of various strains of coli bacteria that as we know still plays a special role in the intestinal diseases of babies, undoubtedly originated with Escherich" (38, p. 723). Escherich's "observation on intestinal bacteria in young children at once became a classic and fundamental work" (19, p. 52). "He certainly deserves credit for realizing how important a role bacteria play in gastrointestinal disorders in infancy. He demonstrated that this normal inhabitant of the intestinal tract [*Bacterium coli*] could become pathogenic and virulent .... The bacterial era [was] inaugurated in pediatrics by Escherich" (4, pp. 114–115). "As long as Escherich lived, the bacterial flora of the gastrointestinal tract was his favorite topic of study. Frequently, when a foreign student came to the clinic eager to study a problem, Escherich would suggest his pet subject" (4, p. 115). Although Escherich's interest and researches subsequently branched out in many different directions, he did not lose his enthusiasm for his first chosen field. He recognized many strains of *Bacterium coli commune*, differing in morphology and biological behavior, and initiated the notion of their pathogenicity. Far in the future lay the insights into enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, and enteroadherent *E. coli* (EPEC, ETEC, EIEC, EHEC, and EAEC) (cf. 74, p. 413; 75, 76, 77, 78, 79, pp. 45–46, 120–134, 491–503; 80, 81), especially EHEC O157:H7, the main culprit in what has been described as Hamburger Disease or Barbecue Syndrome. "What became of the pathogenic strains that were discovered by Escherich? Today fifty million children still die world-wide of diarrheas caused by them" (82, p. 708). The versatility of *E. coli* has gone much further: It "has been incriminated in infections of almost every human organ system" (cf. 83, 84, p. 144) (cf. 85 for a review on *E. coli* with a pediatric emphasis).

Escherich's Vienna Hospital: a Mecca for Pediatricians

Escherich's intense interest in research and his fine and pleasant personality (60, p. 266) made the Vienna St. Anna Children's Hospital a "Mecca for pediatricians" (4, p. 114). It has been stated that Escherich's clinic exceeded any other scientific training institute in the number of university professors and hospital directors that it produced, both in Germany and in Austria (86). As pointed out by his brother-in-law, Meinhard von Pfaundler, in his obituary: "Escherich did not recognize his limits, only the urge to more intensive living, to stronger fight, to more work. He could not think that even his amazing energy could give out, that anything could interfere that was stronger or more powerful than his will to create" (33).

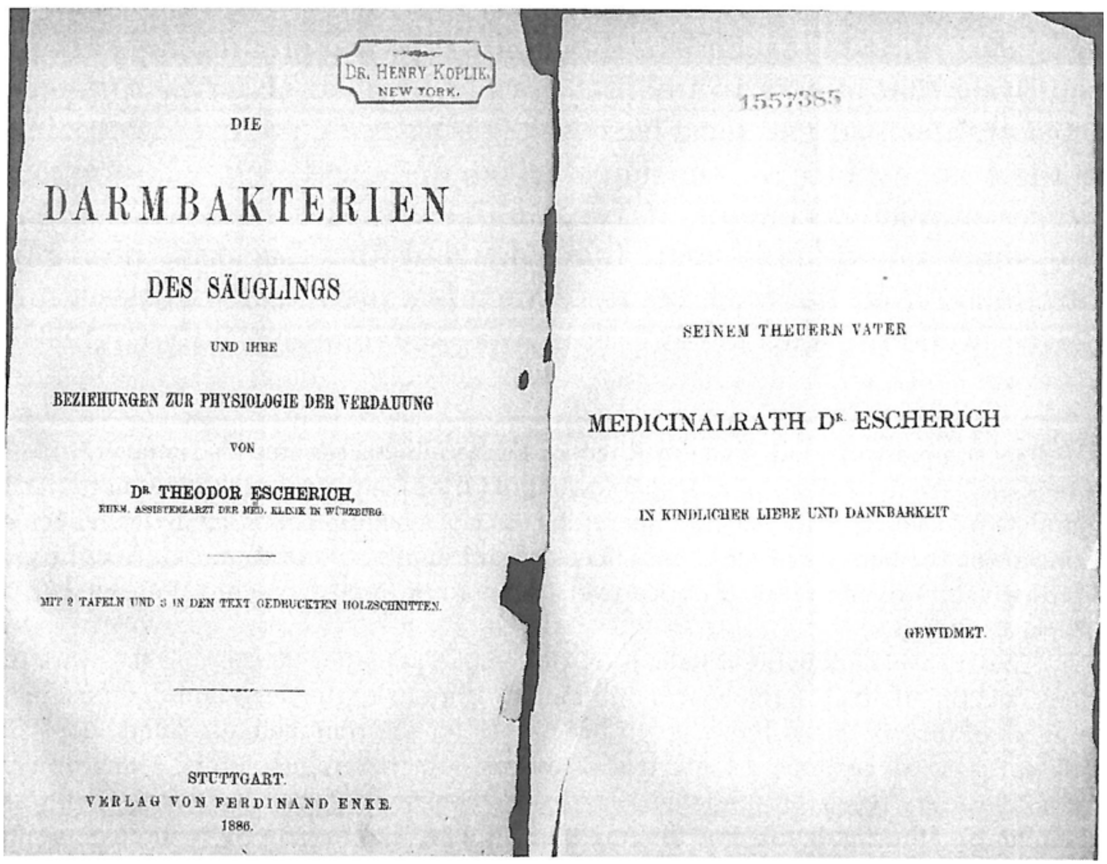
Escherich's Books and a Review

In addition to his first book at age 29, on intestinal bacteria (13) (Fig. 2), which included classic descriptions of the two bacteria that he named *Bacterium coli commune* and *Bacterium lactis aerogenes* (later called *Bacterium aerogenes*, often confused with *Enterobacter aerogenes*, and now called *Klebsiella pneumoniae*, cf. 87, p. 1007), he published three books or monographs, on epidemic diphtheria (1894), diphtheria, croup, and serum therapy (1895), and on tetany in children (1909), and with his brother-in-law, Meinhard von Pfaundler, a lengthy review of the impressive knowledge of *Bacterium coli commune* that had built up in the 18 years since its discovery (88). The review ends with about 600 references to authors, most by now forgotten but an indication of the extent of work and interest in this field.

Escherich's Clinical Versatility

In Graz and then in Vienna, Escherich built schools of pediatrics. Many of his associates moved with him from Graz to Vienna; among them were Béla Schick and Clemens von Pirquet. It has been stated that there was not a topic in pediatrics in which Escherich was not interested (89) and to which he did not contribute. He coined the term idiopathic tetany and established its parathyroid source (cf. 38, p. 724) long before the subject of endocrine secretions was known.

He was one of the first, with Klemensiewicz, to show the presence of antitoxins in the serum of children who had spontaneously recovered from diphtheria (54). He instituted antitoxin therapy in his clinic patients. He vigorously sponsored Paul Moser's antistreptococcus serum in scarlet fever treatment. Although he failed to show a direct relation between *Bacterium coli commune* and diarrhea, he was the first to show that it brought about bladder infection (cf. 90, p. 357). Some have regarded


DR. HENRY KOPLIK. NEW YORK.

DIE

DARMBAKTERIEN

DES SÄUGLINGS

UND IHRE

BEZIEHUNGEN ZUR PHYSIOLOGIE DER VERDAUUNG

VON

Dr THEODOR ESCHERICH,

EHEM. ASSISTENZARZT DER MED. KLINIK IN WÜRZBURG.

MIT 2 TAFELN UND 3 IN DEN TEXT GEDRUCKTEN HOLZSCHNITTEN.

STUTTGART.

VERLAG VON FERDINAND ENKE.

1886.

1557385

SEINEM THEUERN VATER

MEDICINALRATH Dr ESCHERICH

IN KINDLICHER LIEBE UND DANKBARKEIT

GEWIDMET.

Figure 2 Title and second page of Theodor Escherich's classic book, 1886, "The Infant's Intestinal Bacteria and their Relationships to the Physiology of Digestion," to "His dear father, Senior Medical Officer (Medizinalrath) Dr. Escherich, dedicated with a child's love and gratitude." Copyright 2006, Elsevier, Inc. http://www.sciencedirect.com/science/article/pii/S0065216406600051 doi:10.1128/ecosalplus.ESP-0025-2013.f2 this to be among his most important discoveries (cf. 33, p. 522). "The discovery of colicystitis and *Streptococcus enteritis* of infants is exclusively the result of Escherich's researches" (91, p. 750). "He was intensely interested in the diagnosis, pathogenesis, and control of tuberculosis. He pioneered in X-ray detection of the disease in children" (69, pp. 404–405). In 1889, he confirmed the causal role of the Klebs-Löffler bacillus in a diphtheria epidemic. He made important contributions to the study of infant feeding. His name is associated not only with *Escherichia coli* but also with Escherich's reflex (manifested by a muscular contraction of the lips) (92; cf. 93). Escherich's name appears in the most unexpected places, a further indication of his versatility. Thus, a compilation of classical papers in clinical dermatology (94, pp. 331–332) included his 1904 paper on erythema infectiosum (95). "He narrowly missed discovering dysentery bacilli, of which he isolated several cultures, only to discard them because they failed to produce gas in carbohydrate-containing media" (69, p. 404).

Escherich as Teacher, Pediatric Scholar, and Master Educator

In addition to his direct clinical contributions, he "had no equal in his time, either as a teacher and scholar, or as an organizer" (19, p. 83). He gave stimulating lectures (69, p. 404), was an enthusiastic and conscientious teacher, and excelled in describing the pathology of the various diseases of children (60, p. 266). As an educator, Escherich was member of an 1892–1893 committee, including the later Nobel laureate Julius Wagner-Jauregg (1857–1940), that separated the study of medicine into a preclinical and a clinical section and that required a preliminary examination in general biology rather than in individual sciences such as mineralogy, botany, and zoology. It took many years, in fact until 1899, for this suggestion to be adopted (96, p. 268). The fame of the Graz children's hospital expanded well beyond the borders of Austria. The Vienna children's hospital was one of the oldest and most prestigious in Europe. The hospital constructed according to his plans, but which he did not live to see completed, was considered after World War I to be the most beautiful and the largest in the world (70, p. 198).

Escherich in the course of his career published about 190 papers in addition to his books, and he supervised 271 papers of his associates, whose names were always cited as first authors or by themselves. He served as head of the German pediatric society. As Pfaundler stated in his Escherich obituary (33), it was hard to imagine German pediatrics without Escherich. His work in pediatrics and in bacteriology was so wide-ranging that it is hard to do justice to all of it. In fact, on comparing various obituaries and later biographical sketches, it is clear that many do not mention some of his diversified activities. A summary is indicated to give a notion of the range of his interests and contributions apart from his discovery and work with *Escherichia*. An excellent brief survey is given by Fischer (97, p. 375).

Escherich's Sudden Death

Escherich died at the comparatively young age of 54. The death of his son Leo from appendicitis some 5 years earlier had strongly affected him with increasing signs of arteriosclerosis (cf. 98). "The first symptom of the impending disaster was that Escherich started to talk French at the rounds and complained of headache. He died suddenly several days afterward, apparently from a cerebral hemorrhage" (4, p. 122). A more sedate version is given in the obituary in *The Lancet* (99): "Suddenly he began to speak in different languages ... and he had to be conveyed to his home, where he died the next day." According to Wagner (100, p. 87), however, Escherich died during a lecture to his students.

Summary: The Life of a Socio-Pediatrician

Escherich's name should be remembered not only as the discoverer of the bacterium named after him, but as one of the preeminent pediatricians of his time, head of one of the most respected pediatric clinics whose renown he furthered, tireless contributor to a variety of medical and social fields, prolific author of books, monographs, and papers, and outstanding medical educator (cf. 97, 101). A pioneer pediatrician who devoted his efforts to improving child care, particularly infant hygiene and nutrition (101), "Escherich believed that pediatrics consisted not only of research and the cure of diseases, but also of prophylactic work" (91, p. 750). He has aptly been called a socio-pediatrician (102). "Less versatility and longer life might have won him greater celebrity and more durable renown" (69, p. 405).

Obituaries

Escherich's work extended over the whole area of pediatrics, both in terms of clinical work and in terms of strong social interests in the welfare of children and their mothers. In accord with this diversity, contemporary appraisals, evident most clearly from his 1911 obituaries, select one or other of his accomplishments, but most decidedly do not stress his work on children's digestive disorders. Escherich's eminence and unexpected death at an early age stimulated an immediate outpouring of obituaries in leading international medical journals (21, 33, 60, 86, 99, 103, 104, 105, 106, 107). The one from the United States stated "His professional reputation was international" (103). The various writers agreed that he contributed to all regions of pediatrics. They selected different aspects of his medical achievements for special praise such as his studies on tetany, diphtheria, tuberculosis, cystitis, and nutrition. Recurring emphases, apart from his prolific medical contributions, were his "tremendous energy for work" (103), "a force of nature with superhuman capacity for work" (60, p. 266), his renown as a teacher, his great organizing skill, manifested particularly in his detailed planning of the splendid new children's hospital in Vienna and in his perseverance in establishing an organization, probably far ahead of its time, whose purpose was to help infants in need of proper nutritional help (Säuglingsschutz). Many of the obituaries, written under the direct spell of his strong personality, emphasized that his unusual ability to apply bacteriology to pediatrics made him the father of bacteriological pediatrics. Some of these evaluations mention but do not especially emphasize what later insight has selected as his most important contribution, the discovery of *Bacterium coli commune*.

A comparison of the recurring tenor of contemporary Escherich obituaries with the numerous appraisals of his work published on the centenary of his birth is highly instructive. Past fame and present acceptance do not always run in parallel. Much that was in the forefront of awareness by his contemporaries moved into the background some 40 years after his death. Thus in a long, highly emotional obituary by Julius Zappert, written on the day of Escherich's death and published in the prestigious *Wiener Medizinische Wochenschrift* a mere 3 days later, one is impressed by the description of Theodor Escherich's strong personality, his organizing ability, and his wonderful way with children. His founding of the organization "Säuglingsschutz" (Protection of Newborns) for the distribution of mother's milk to needy mothers, his initiation, through sheer hard work, of the Vienna pediatric society, and his tireless work toward the building of a new children's hospital, whose completion he did not live to see, are emphasized. His principal work was stated to have consisted in the founding of a statewide organization for the care of mothers and newborns. Praise is given to the fact that some of his many pupils were in charge of three of the largest pediatric clinics in Germany (in Munich, Breslau, and Heidelberg). As a tribute to his impressive energy and versatility, there is a list of his major clinical accomplishments shown by publications on diphtheria, tetany, streptococcal enteritis, pediatric tuberculosis, the demonstration that bladder infection is caused by *Bacterium coli*, and numerous others. The 1886 book, "The Intestinal Bacteria of Children" (Die Darmbakterien des Kindes) "which reported facts completely new at the time and still accepted" was mentioned to have allowed him to move toward the forefront of pediatricians. However, this work was not discussed any further. Similarly, the three-page obituary by Meinhard von Pfaundler discusses Escherich's work on intestinal bacteria, but his other clinical achievements and his medically related social enterprises are emphasized (33). Clemens von Pirquet, his student and successor in Vienna following Escherich's death, describes him as one of the founders of scientific pediatrics and one of its most important representatives. His detailed obituary ends with the statement "how proud we are of Escherich's scientific discoveries and the social-hygienic organizations that he initiated and that will ensure his name to be conveyed to posterity." As stated in connection with the death of another scientist, "Eulogies and obituaries were a common genre of memorial literature, and many notices, essays and printed speeches appeared in the wake of [one's] death" (108, p. 15).

Late Appraisals of Escherich's Accomplishments

Later writings about Theodor Escherich, insofar as they understandably stressed his bacteriological work, especially his discovery of *E. coli*, give an incomplete and distorted impression of his many and varied accomplishments. Appraisals were published more or less on the occasion of Escherich's centenary (4, 19, 38, 58, 69, 70, 89, 90, 96, 97, 109, 110, 111) and, later, in celebration of the centenary of his discovery of *Bacterium coli commune* (23, 56, 82, 102, 112, 113, 114, 115, 116). These articles are impressive in their number and in their marked change in emphasis, for now *Escherichia coli* has moved inexorably to the fore, and Escherich's social and varied clinical achievements are subjects, if at all, of dutiful but rather transitory recitation.

There is an Escherich street in Vienna (in the 19th Borough), an Escherich Pavilion existed in the pediatric clinic of the University of Vienna (destroyed in World War II), and the Austrian Pediatric Society issued a plaque "for merit" first awarded in the centenary, in 1986, of Escherich's publication of his book on the intestinal bacteria of the newborn (31, p. XI).

Escherich's Uniqueness

Although publications that praise and evaluate great men often ignore comparisons with others, it is useful to do so now. He combined, even at an early age, clinical and rigorously scientific attitudes and pursuits; he manifested a clear recognition of objective knowledge separate from direct clinical observations as a prerequisite to eventual clinical insight. His quest for the bacterial cause of devastating childhood dysentery was made difficult by the well-nigh overwhelming variety of intestinal microorganisms. It demanded a thoroughly analytical approach as a prerequisite for an answer to his motivating question. Although his own work did not lead to an answer, his discoveries led to basic insights into fecal bacteria as related to location within the intestine, and the host's age and nutrition. A less-schooled investigator might have missed or ignored these complex interactions. Escherich was impressive in the thoroughness and patience of his work, in his persistence of seeking the bacterial etiology of childhood dysentery, and in his clear recognition, clearly expressed, that an inability to show a direct connection between the presence of his favorite organism *Bacterium coli commune* and dysentery did in no way render his and many others' studies of this organism irrelevant to the clinical problem at hand. We see here, therefore, an admirable scientific attitude that in addition fertilized his numerous other clinical interests and accomplishments. The masterful descriptions in his 1886 book, a work written in his twenties, stimulated early inquiry and discovery on the part of a host of now forgotten investigators. Escherich was lucky to discover an organism that for sheer ease and rapidity of growth in the test tube ultimately led to fundamental discoveries in bacteriology, biochemistry, and molecular biology of which he had no inklings. In addition to its ready cultivability, Escherich's bacterium lacked the kind of pathogenicity manifested by organisms discovered by his contemporaries, thus stimulating wide-ranging interest into its biology, more and more divorced from its role as an intestinal organism. Luck is not a quality of accomplishment that denigrates a person's renown. As Paul Ehrlich said in an oft-quoted passage, scientific success needs patience, skill, money, and luck (Geduld, Geschick, Geld, und Glück) (cf. 117, p. 24). Escherich's versatility and scientific focus deserve one's attention and one's admiration. He made substantial contributions to a variety of medical fields, upheld the social imperatives of his discipline, and possessed an impressive organizational ability. His pleasant and yet inspiring personality, so highly appreciated during his life, helped Escherich's achievements beyond his early clinical and bacteriological observations.

FIRST STUDIES WITH *BACTERIUM COLI COMMUNE*: SEARCH FOR THE BACTERIAL CAUSE OF INFANTILE DIARRHEA

Introduction

Escherich's Naples experiences on cholera stimulated his investigations of children's feces and infantile diarrhea immediately on his return from Naples. In every one of the known, recently discovered pathogens, and many still to come, there was a direct correlation between a given disease and a given pathogen. It was unusual that Escherich did not isolate the causative organism. His motivation was the same, but it developed quite differently. Dysentery was an ancient disease. "[...] the Latin word *pestis*, which was widely used until the seventeenth century, was used to indicate any of the great epidemic diseases, such as plague, typhus, smallpox, or dysentery." "The first book of Samuel in the Old Testament provides the earliest detailed description .... Although all retrospective diagnoses are speculative, the disaster was probably an outbreak of dysentery" (118, 11. 6–7). An epidemic in 580 was described by Gregory, Bishop of Tours, and in 1670 Thomas Sydenham described it as causing "great torment of the bowels" (119, p. 28). Escherich hoped that a bacteriological approach would lead to a discovery of the cause of this decimating affliction. He faced a daunting challenge: in contrast to the clear-cut cause and effect relationship presented by other pathogenic bacteria, the unraveling of the cause of dysentery presented a different picture. As he said in an 1885 lecture:

> At a time when Koch's experimental methods reap such rich laurels in the newly revealed fields of the etiology and pathology of infectious diseases it might appear to be a useless and almost thankless task to attempt to disentangle the apparently completely unregulated mass of intestinal bacteria, dependent on a thousand contingencies, of intestinal bacteria in normal stools. When, however, I have by now devoted a year almost exclusively to this special study, I did this in the conviction that the exact knowledge of these conditions is essential not only for

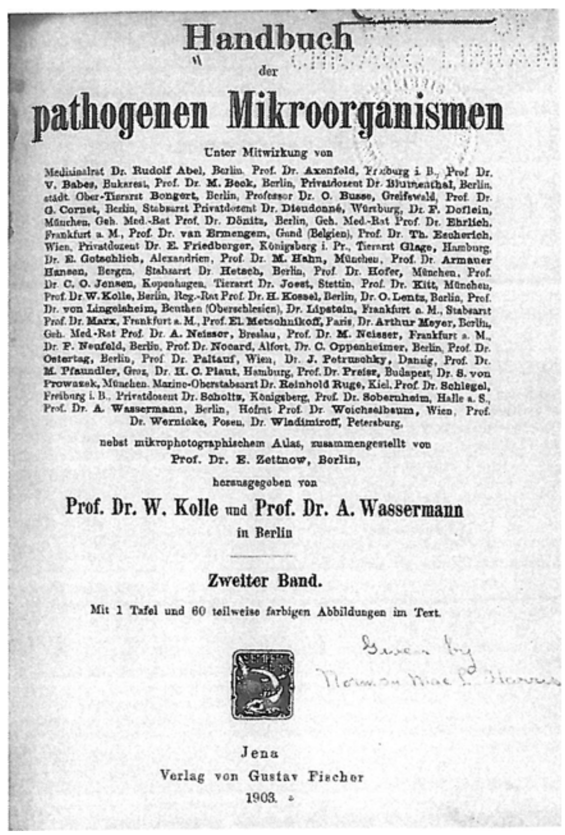
> the physiology of digestion where intestinal putrefaction remains an unknown and incalculable X, but also for the pathology and therapy of the bacterial intestinal diseases.
>
> (Escherich [120] quoted by Kundratitz [38])

The approach of the author, from a study of normal conditions to a study of pathology and from microscopy to bacterial cultures, has a thoroughly modern flavor. We find a balance between the attitudes of the pure scientist and the clinician, and an impressive mastery of both.

Escherich's publications leading to *Bacterium coli commune* began with a talk in Munich to the Society for Morphology and Physiology, 17 December 1884 (121). He reported preliminary studies indicating that infants in the first days of life had about 20 different types of intestinal and fecal bacteria. The bacteria, grouped according to their appearance in the microscope, included colored sarcinae, 5 types of micrococci, and 10 types of bacilli. No names were given. The short report ends with the statement that bacteria, probably introduced from the food, could be demonstrated in the feces within 12 to 24 hours after birth. This summary of a talk given by Theodor Escherich at a session, 17 December 1884, of the Gesellschaft (Society) für Morphologie und Physiology at Munich is so short that a complete translation is given here: Escherich (121) Ueber die Bakterien des Milchkotes (Concerning the bacteria of milk feces [feces of children fed mother's milk]). Aerztliches [Medical] Intelligenz-Blatt [Publication], Münchner Medicinische Wochenschrift [Weekly] **32:**243.

"The speaker, following a Referat by professor Tappeiner concerning the work of Bienstock, reports that in the course of his investigations of the feces and the intestinal contents of infants in the first days of life he has for the time being isolated about 20 different types of bacteria: 2 tooth types, several colored sarcinae forms, 5 micrococci and 10 bacillary types. Of the latter, 5 types are constantly found in large amounts as inhabitants of the duodenum and the small intestine, while they appear to form spores in the less suitable conditions encountered in the colon and in the rectum. The occurrence of the micrococci is limited to the large intestine, while that of the sarcinae is restricted to the lowermost part of the rectum. For the latter the entry per anum into the intestinal canal has been established, while the bacilli and cocci are probably introduced with the food and can be detected in the feces as early as 12–24 hours after birth."

This was the opening salvo, greatly developed in four subsequent publications that inexorably paid more and more attention to *Bacterium coli commune*: a long 14 July 1885 lecture (120) before the same society; a year later his celebrated book on the intestinal bacteria of children (13) (Fig. 3); a historical survey in 1887 on intestinal bacteria and diseases (122); and in 1903, with Pfaundler (cf. 123, p. 97), a highly detailed 141-page review including 21 pages of densely printed references. The latter was the very first article dealing exclusively with *Bacterium coli commune*. This review, just short of 20 years after the discovery of the organism, was part of the Handbook of Pathogenic Microorganisms (Fig. 4), with articles by Victor Babes, Paul Ehrlich, Armauer Hansen, Élie Metchnikoff, and Albert Neisser. Escherich added new observations in each of his various publications. The cumulative effect was one of concentrated hard work, elegance, and critical familiarity with the latest contributions to an exploding field of medicine and bacteriology.



Handbuch
der
pathogenen Mikroorganismen

Unter Mitwirkung von

Medizinalrat Dr. Rudolf Abel, Berlin. Prof. Dr. Axenfeld, Freiburg i. B., Prof. Dr. V. Babes, Bukarest, Prof. Dr. M. Beck, Berlin, Privatdozent Dr. Blumenthal, Berlin, städt. Ober-Tierarzt Bongert, Berlin, Professor Dr. O. Busse, Greifswald, Prof. Dr. G. Cornet, Berlin, Stabsarzt Privatdozent Dr. Dieudonné, Würzburg, Dr. F. Doflein, München, Geh. Med.-Rat Prof. Dr. Dönitz, Berlin, Geh. Med.-Rat Prof. Dr. Ehrlich, Frankfurt a. M., Prof. Dr. van Ermengem, Gand (Belgien), Prof. Dr. Th. Escherich, Wien, Privatdozent Dr. E. Friedberger, Königsberg i. Pr., Tierarzt Glage, Hamburg, Dr. E. Gotschlich, Alexandrien, Prof. Dr. M. Hahn, München, Prof. Dr. Armauer Hansen, Bergen, Stabsarzt Dr. Hetsch, Berlin, Prof. Dr. Hofer, München, Prof. Dr. C. O. Jensen, Kopenhagen, Tierarzt Dr. Joest, Stettin, Prof. Dr. Kitt, München, Prof. Dr. W. Kolle, Berlin, Reg.-Rat Prof. Dr. H. Kossel, Berlin, Dr. O. Lentz, Berlin, Prof. Dr. von Lingelsheim, Beuthen (Oberschlesien), Dr. Lipstein, Frankfurt a. M., Stabsarzt Prof. Dr. Marx, Frankfurt a. M., Prof. El. Metschnikoff, Paris, Dr. Arthur Meyer, Berlin, Geh. Med.-Rat Prof. Dr. A. Neisser, Breslau, Prof. Dr. M. Neisser, Frankfurt a. M., Dr. F. Neufeld, Berlin, Prof. Dr. Nocard, Alfort, Dr. C. Oppenheimer, Berlin, Prof. Dr. Ostertag, Berlin, Prof. Dr. Paltauf, Wien, Dr. J. Petruschky, Danzig, Prof. Dr. M. Pfaundler, Graz, Dr. H. C. Plaut, Hamburg, Prof. Dr. Preisz, Budapest, Dr. S. von Prowazek, München, Marine-Oberstabsarzt Dr. Reinhold Ruge, Kiel, Prof. Dr. Schlegel, Freiburg i. B., Privatdozent Dr. Scholtz, Königsberg, Prof. Dr. Sobernheim, Halle a. S., Prof. Dr. A. Wassermann, Berlin, Hofrat Prof. Dr. Weichselbaum, Wien, Prof. Dr. Wernicke, Posen, Dr. Wladimiroff, Petersburg,

nebst mikrophotographischem Atlas, zusammengestellt von
Prof. Dr. E. Zettnow, Berlin,

herausgegeben von

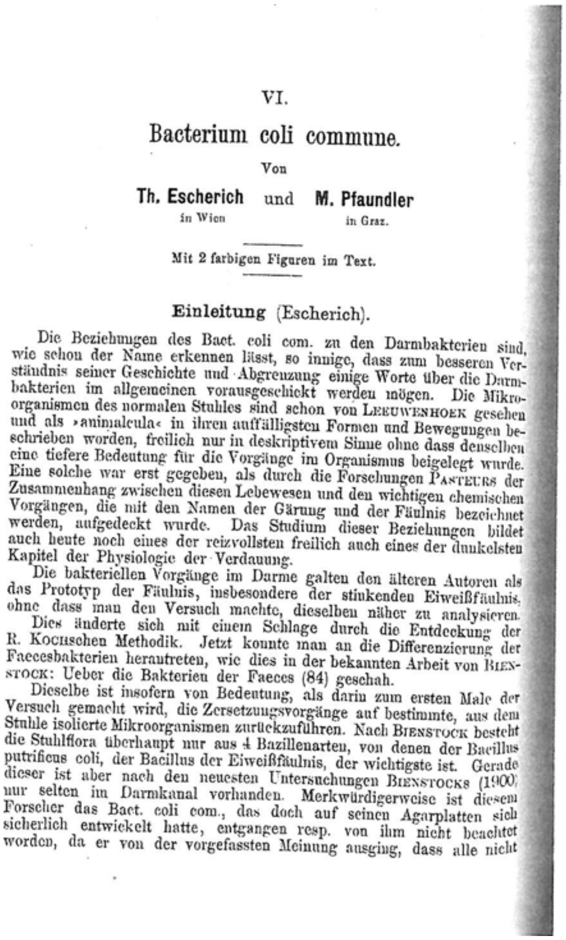
Prof. Dr. W. Kolle und Prof. Dr. A. Wassermann
in Berlin

Zweiter Band.

Mit 1 Tafel und 60 teilweise farbigen Abbildungen im Text.

Jena
Verlag von Gustav Fischer
1903.

Figure 3 Title page, volume two, of the four-volume comprehensive "Handbook of Pathogenic Microorganisms," 1903, edited by W. Kolle and A. Wassermann. Copyright 2006, Elsevier, Inc. http://www.sciencedirect.com/science/article/pii/S0065216406600051 doi:10.1128/ecosalplus.ESP-0025-2013.f3



VI.

Bacterium coli commune.

Von

Th. Escherich in Wien und M. Pfaundler in Graz.

Mit 2 farbigen Figuren im Text.

Einleitung (Escherich).

Die Beziehungen des Bact. coli com. zu den Darmbakterien sind, wie schon der Name erkennen lässt, so innige, dass zum besseren Verständnis seiner Geschichte und Abgrenzung einige Worte über die Darmbakterien im allgemeinen vorausgeschickt werden mögen. Die Mikroorganismen des normalen Stuhles sind schon von Leeuwenhoek gesehen und als »animalcula« in ihren auffälligsten Formen und Bewegungen beschrieben worden, freilich nur in deskriptivem Sinne ohne dass denselben eine tiefere Bedeutung für die Vorgänge im Organismus beigelegt wurde. Eine solche war erst gegeben, als durch die Forschungen Pasteurs der Zusammenhang zwischen diesen Lebewesen und den wichtigen chemischen Vorgängen, die mit den Namen der Gärung und der Fäulnis bezeichnet werden, aufgedeckt wurde. Das Studium dieser Beziehungen bildet auch heute noch eines der reizvollsten freilich auch eines der dunkelsten Kapitel der Physiologie der Verdauung.

Die bakteriellen Vorgänge im Darme galten den älteren Autoren als das Prototyp der Fäulnis, insbesondere der stinkenden Eiweißfäulnis, ohne dass man den Versuch machte, dieselben näher zu analysieren.

Dies änderte sich mit einem Schlage durch die Entdeckung der R. Kochschen Methodik. Jetzt konnte man an die Differenzierung der Faecesbakterien herantreten, wie dies in der bekannten Arbeit von Bienstock: Ueber die Bakterien der Faeces (84) geschah.

Dieselbe ist insofern von Bedeutung, als darin zum ersten Male der Versuch gemacht wird, die Zersetzungsvorgänge auf bestimmte, aus dem Stuhle isolierte Mikroorganismen zurückzuführen. Nach Bienstock besteht die Stuhlflora überhaupt nur aus 4 Bazillenarten, von denen der Bacillus putrificus coli, der Bacillus der Eiweißfäulnis, der wichtigste ist. Gerade dieser ist aber nach den neuesten Untersuchungen Bienstocks (1900) nur selten im Darmkanal vorhanden. Merkwürdigerweise ist diesem Forscher das Bact. coli com., das doch auf seinen Agarplatten sich sicherlich entwickelt hatte, entgangen resp. von ihm nicht beachtet worden, da er von der vorgefassten Meinung ausging, dass alle nicht

Figure 4 First page of Escherich and Pfaundler's 141-page review on *Bacterium coli commune* in the 1903 Handbook. Escherich's introduction mentions Leeuwenhoek, Pasteur, and Koch. Copyright 2006, Elsevier, Inc. http://www.sciencedirect.com/science/article/pii/S0065216406600051 doi:10.1128/ecosalplus.ESP-0025-2013.f4

The 1885 Lecture

In the very first sentence of the 1885 lecture, translated 100 years later into English (124), Escherich stated that it may appear pointless and thankless to disentangle the myriad bacteria that occur in the stools and in the intestinal canal, but that he was not going to give up (120). The year spent on this topic, to the exclusion of almost everything else, was motivated by the expectation that studies of these bacteria could lead to a better understanding (i) of the physiology of digestion and (ii) of the pathology and therapy of microbial intestinal diseases. He emphasized the importance of the latter topic since the mortality from intestinal diseases continued to decimate the world of infants (unsere Säuglingswelt) with unchanged ferocity. His studies did answer some questions concerning microbial processes in the digestive tract but did not identify the bacterial etiology of intestinal diseases. Nevertheless, his work paid off in other ways. With impressive thoroughness he embarked on a study of the bacterial population found in the intestinal excreta, right from birth. He discovered that within the first 3 days of life there were three distinct bacterial profiles. The first of these profiles was shown by the meconium, the third by the subsequent feces. At the very start of life, the meconium was sterile, but, in the first profile, a variety of bacteria and yeasts sometimes was present as soon as 4 to 7 hours after birth.

Three Bacterial Profiles in Intestines of the Developing Infant: Prevalence of *Bacterium coli commune* in Early Life

Escherich described a number of these bacteria, including some spore-formers, and others that occurred in smaller numbers and that could be grown in culture, including *Bacillus subtilis* and the rods that he called *Bacterium coli commune*. A dense microbial population developed after about 24 hours. He inferred that this population entered through the mouth with the very first breaths of air and also with air via the anus, since the population varied with the dust content of the air, the temperature, and the time of year. In milk-fed infants he found a sudden change in the bacterial profile, an observation of primary importance in the study of *Bacterium coli commune*.

In the second profile, the earlier diverse bacterial population had been replaced by a single bacterial type, consisting of slender, at times slightly bent, short rods that appeared to be a pure culture. He called it *Bacterium coli commune*. He gave a detailed description of these bacteria, noted over 100 years later to be up-to-date (23, p. 256). He emphasized their polymorphism, also observed in cultures, although this disappeared after a number of subcultures on a gelatin medium. He described the appearance of colonies grown on media with agar, blood serum, potatoes, milk, and cane sugar. This thoroughness was accorded to only one of the other bacteria, described almost as an afterthought, which he called *Bacterium lactis aerogenes*. (His concepts were not too clear, since he talked of a "pure *Bacterium coli commune* culture" and then mentioned *B. lactis aerogenes*, admittedly present in smaller amounts.)

In a 1900 study, Ernst Moro, one of Escherich's students, confirmed Escherich's observations on the distinctions between the bacteria of meconium feces and mother's milk feces and extended these distinctions to cow's milk feces (125). He made two interesting points: he used the adept phrase "the vegetation from meconium feces to mother's milk feces changes with one blow" (ändert sich mit einem Schlage), and he indicated that an examination of the baby's feces permitted one to determine even an occasional administration of cow's milk.

The third profile, with its variety of microorganisms, tended to resemble that of the adult.

Escherich's Classical 1886 Book on Infants' Intestinal Bacteria: Masterpiece of a 29-Year-Old

In his classical 1886 book on the intestinal bacteria of infants, Escherich addressed three related questions: the normal bacterial composition of the intestinal tract of infants and the changes that these undergo right from birth, the role in nutrition of the decomposition of foods by intestinal bacteria, and the relation of these studies to pathological conditions. There had been but one study on microscopic examinations of feces of normally fed infants, by Uffelmann in 1881 (126), and there had been very few attempts at cultivation of intestinal bacteria from adults by the new methods of Koch. Escherich emphasized that microscopy would furnish only the framework for studies that used bacterial cultures. In contrast to Uffelmann, who detected but two different bacteria in infant's stools with microscopic observation, Escherich succeeded in isolating at least 19 different bacteria from the intestinal contents of infants and carefully described their morphology and cultural requirements (cf. 116, p. 8). He paid special attention to the regularly occurring and numerically predominant *Bacterium coli commune*. He demonstrated that these bacteria could grow in the absence of oxygen, that is, under conditions resembling those found in the intestine (cf. 31, p. 313). He stated: "They were thus-far found only in the intestinal canal, especially in its lower parts, and therefore they were called 'colon bacteria'" (13).

A brief summary of this work provides a rich insight into the background motivation, results, and outlook of Escherich's investigations of children's feces. He spent 15 months in this new enterprise, away from his exclusively clinical work, spending days and nights sitting over his microscope. He started his book by pointing out that the first to show the occurrence of the "smallest living beings" in the feces was Leeuwenhoek, in a 1719 letter to Robert Hooke: "de vivis animalculis existentibus in excrementis" (127). In a footnote, Escherich gave a long passage from Leeuwenhoek in Latin, without translation, indicative of his own learning and of his assumption that his readers would not need a translation. In a learned article Dobell indicates, without reference to or apparent knowledge of the 1719 letter, that Leeuwenhoek, "the first to observe the intestinal protozoa of man," recorded his discovery in 1681 from his own stools (128, p. 1), and that it can be concluded from his detailed description that he observed *Giardia* (=*Lamblia*) *intestinalis* (128, p. 15). Dobell makes the point that all of Leeuwenhoek's letters were written in Dutch, "the only language which he could read or write" (128, p. 3), and that he discovered free-living protozoa earlier than 1675 (128, p. 1). Dobell (129, p. 198) quotes the eminent Dutch microbiologist Beijerinck to the effect that Leeuwenhoek's "animalcules" "were undoubtedly bacteria —not protozoa—and that among them were probably (as he found in his own experiments) *Bacillus coli, Azotobacter*, and *Amylobacter saccharobutyricum*" (130).

Escherich was very much aware of the distinction to be made between the bacterial picture revealed by direct examination with the microscope and subsequent attempts at bacterial cultures, for not all bacteria will grow in culture. He described in detail his invention of a painless and effective method to remove fecal material from babies, using aspiration with a syringe under sterile conditions. He stressed the difference between the miniscule bacterial content of feces from babies obtained up to 14 hours of birth (meconium feces) and that obtained on milk feeding (milk feces). The meconium feces were sterile or sparsely populated (he described three bacteria, *Proteus vulgaris, Streptococcus coli gracilis*, and *Bacillus subtilis*), while the milk feces had an abundance and variety of bacteria, different from those in the meconium feces. Escherich divided these bacteria into two different kinds, *obligate* and *facultative*. The obligate bacteria, always present in large amounts, consisted of just two novel species, which he named *Bacterium lactis aërogenes* (former spelling) and, present in far greater abundance, *Bacterium coli commune* (cf. 120). As to the facultative fecal bacteria, Escherich described 14 different species. These occurred in much smaller numbers and were not always present. Of these, only one, *Micrococcus ovalis*, had a distinctive name. As a sign of bacterial classification at the time, four yeast species (*Torula* and *Monilia candida*, as well as "red yeast" and "capsula yeast") were included. Escherich discussed at great length the relation between facultative intestinal bacteria and anaerobiosis. He was very much aware of facultative anaerobiosis as a possible explanation for the rather limited number of species found in the intestine. He described a device that he designed to grow bacteria anaerobically. *Bacterium lactis aerogenes* and *Bacterium coli commune*, especially the latter, got by far the most attention. This organism was found from 14 hours after birth but not before. He demonstrated that these two organisms had different pathogenic activities in different animals. On injections in various ways, he found that *Bacterium coli commune* was highly pathogenic in guinea pigs, pathogenic in cats, less so in rabbits, and nonpathogenic in mice and dogs. He performed detailed autopsies on the animals that succumbed. He observed that *Bacterium lactis aerogenes* manifested a similar distribution of pathogenicity. He emphasized the polymorphous character of *Bacterium coli commune* and regarded it as almost certainly identical to Brieger's bacillus, isolated from feces (131, 132, 133). He made it clear that *Bacterium coli commune* occurred in the lower part of the small intestine and *Bacterium lactis aerogenes* occured in the upper part. He emphasized (13, p. 39) that not all the fecal bacteria observed microscopically will grow after inoculation on growth medium with gelatin or with agar-agar.

Escherich stressed that the occurrence of bacteria in feces was a perfectly normal phenomenon. In addition, he recognized, without providing data, that the bacterial composition of adults' feces was very different from that of children. He asked whether the facultative organisms, often obtained from children who showed slight digestive symptoms, might be disease related, but he admitted that more clinical and animal experiments were needed to answer this question. The book is impressive in its thoroughness and range and in its bacteriological and pathological acumen. Moreover, the book is largely a piece of scientific research, with only a few statements as to its possible or hoped for medical promises. Thus, on pp. 53–54 one reads:

> Among the endless number of facultative intestinal bacteria I have described in some detail only a few of the more frequently occurring types and groups. Among these we will ... encounter a predominant number of cocci. The relevant types of rods and spout types (*Sprosspilzarten*) were obtained from children who suffered or who had suffered from light digestive disturbances. Did these forms have an etiological relation to the disease? There are various reasons that made it appear very likely that some intestinal diseases of infants are caused by certain microorganisms, and that among the facultative intestinal bacteria some will be found that, starting from the intestine, will bring about disease. However, it is obvious that only the systematic investigation of suitable clinical cases and animal experiments will answer these questions.

The book ends with the following evocation (p. 177):

> The first and most essential basis of further progress ... consists in the study of the physiological processes of fermentation and of the bacteria occurring in the intestine under normal conditions, as has been the aim of the present work. May the perspectives gained here not remain without use and without practical applications on behalf of the therapy of the most murderous pest of the first year of life, the mycotic intestinal diseases.

Rietschel and Hummel (134, pp. 1006–1007) pointed out that Escherich's studies on the intestinal flora should be remembered not only for the discovery of *B. coli commune* and *B. lactis aerogenes* but in addition for stressing the importance of processes of fermentation and putrefaction in infants, for being the first to indicate the relation between nutrition and the bacteria flora, and for initiating studies on the impressive differences in the bacterial flora of different parts of the intestinal canal.

In the part of the book that discussed the properties of *Bacterium lactis aerogenes* and *Bacterium coli commune* in detail (microscopic behavior, growth on a variety of media, macroscopic appearance of colonies, fate of injection into various animals such as guinea pigs, rabbits, cats, dogs, and mice), the former organism was treated in 6½ pages but the latter received 11 pages. Few, if any, animal experiments were carried out with the remaining bacterial types that could be cultured.

The second part of the book dealt with the oxygen consumption of intestinal bacteria. At least 55 experiments were carried out on facultative anaerobiosis of various microorganisms on various media, intended to throw some light on the possible presence of oxygen in the intestinal canal and on the manner by which the intestinal contents were colonized. The third part of the book discussed the physiology of intestinal fermentation in infants, recognized the limited breakdown of proteins by the intestinal bacteria, investigated the acidity of infants' feces, reported experiments on the origin and types of intestinal gases, and discussed the relation between the activities of intestinal bacteria and nutrition. The shortest and last section, "Clinical-Therapeutic Considerations," a mere 4½ pages, reflected the profound lack of knowledge in this area. The book's last sentence stated: "May the views that have been obtained here not remain without use and practical application for the therapy of the most murderous plague of the first year of life: the bacterial infectious diseases."

Escherich's work, undoubtedly facilitated by the application of Koch's bacteriological methods and buttressed by persistence and impressively hard work, brought the knowledge of intestinal bacteria to a new level. The book impresses one by the application of pure science in the search for a practical solution to a serious clinical problem.

From the welter of intestinal forms that he was the first to describe, Escherich extracted both descriptive and, to some extent, interpretative order. This achievement was in no small measure due not only to the patience and thoroughness that he brought to his 1886 book but also to his decision to ignore those organisms that could not be cultured by measures such as the ones newly introduced by Robert Koch. In addition to *Bacterium coli commune* and *Bacterium lactis aerogenes*, he tested his organisms for growth not only on agar media but on gelatin media and on potatoes (remarking that certain types of potatoes were more suited than others), as well as on liquid media. He much preferred gelatin media over agar media. In addition, he instituted clinical tests with *Bacterium coli commune* and *Bacterium lactis aerogenes* and observed that both of the above were on occasion pathogenic in humans and, not always reproducibly, in animals such as guinea pigs, rabbits, cats, and dogs. Escherich made it his task to attempt making some order out of the abundance of bacteria present in infant feces after a few days of life. His persistence and sheer hard work are obvious. He focused on those bacteria that could be cultivated after initial microscopic observation. He distinguished between "the types that were constant inhabitants of the intestinal tract and the ones that were rarer and present in smaller amounts" (p. 53). Escherich's initial aim, to discover a bacterial cause for children's diarrhea, was not satisfied, but in its place a matter of at least equal importance came to light, an emphasis on nutritional factors related to children's well being and disease. Escherich initiated the study of nutritional disturbances as related to health (135, p. 14).

Early Fascination and Denigration of *Bacterium coli*: Lessons of Methodological Limitations

The 1885 discovery of *Bacterium coli commune* initiated the field of intestinal pathology. There is a paradox in this story. The limitations of methods of bacterial cultivation at the time pushed this readily grown and undemanding bacterium to the forefront of interest (cf. 136, p. 111). Friedrich von Müller denigrated the importance of *Bacterium coli*. He emphasized that this organism always pops up when the usual methods of bacterial culture were used, although it represented only a small part, and not necessarily the most important one, of the bacterial population. He implied, therefore, that *B. coli* was rather a nuisance in the search of possible true intestinal pathogens (von Müller [137] quoted by Barth [136], pp. 111, and by Knoke [138], pp. 111–112). A researcher from London at almost exactly the same time (139) praised the hardiness of the organism as a reason for its apparent intestinal prevalence: "[...] it finds thence a large area of distribution, and as a facultative anerobe of great hardiness and fertility, it can thrive in circumstances where many a more delicate microbe would perish." This view was in direct contrast to the prevailing opinion that, for a long time, regarded *Bacterium coli* as the most important intestinal bacterium (cf. 140). We have here a wonderful object lesson in the limitations that available technology places on the pursuit of knowledge. In the intervening century, the source and possible biological function of this organism has become all but irrelevant, and the very ease of cultivation that catapulted it to initial interest has ensured its continuing usefulness. It often has been remarked that the large number of subcultures of various strains of *Escherichia coli* would render it unsuitable for intestinal growth (cf. 113). Most present-day interest in this organism as a research tool for molecular biology completely ignores its long-distant intestinal provenance. Reminders are provided by its many pathological strains and by its use as an indicator of biological contamination (initiated by 141, 142). We will see below how recognition of *Escherichia coli*'s relatively minor contribution to the bacterial population of the colon constitutes an object lesson in advances in bacteriological techniques.

Luck and the Discovery of *Bacterium coli commune*

In terms of the discovery of *Bacillus coli commune*, Escherich was lucky to have studied the stools of infants, for in adult stools the abundance of this organism is rather less. Reports vary, but nowhere else does one find an incidence even approximating that discovered by Escherich in the stools of just a few day-old infants. Another criterion that Escherich used, as stressed at the start of his book, was capacity to grow in culture the bacteria observed under the microscope. *Bacillus coli commune* turned out to be an exceedingly easy organism to grow.

It was the ready cultivability, along with a short generation time, rather than a recondite interest in human excreta, that right from the start contributed materially to the preponderance of studies with this organism. The term "coli" was intimation of its early history, of direct interest only to the clinicians and pathologists who were saddled with intestinal aberrations, but far indeed from the minds of the myriad researchers who studied bacterial mutants, genes, and clones, and used the organism to unravel the genetic code, bacterial conjugation, DNA and RNA synthesis, topoisomerases, bacteriophages, and the mysteries of gene expression. Strains such as *E. coli* ML or K-12 are progeny distant from even a cursory acquaintance with a colon, far removed from their ancestral progenitors. They would, in fact, not be able to exist in human intestines (cf. 113, p. 65). "Most work on *E. coli* centers around one particular strain, known as Nissle 1917. It was isolated in World War I from a soldier who survived a particularly severe outbreak of diarrhea. Nissle proposed the use of *Bact. coli* as early as 1916 and showed in the 1930s that administration of this strain improved symptoms in patients with non-infectious bowel disorders" (143).

The Incidence of Intestinal *E. coli*: A Tale of Diminution

Since Escherich's days there has been a flowering, if one is permitted to use this term, of interest and research in intestinal flora. The estimated number of species in the human microflora has been increasing from "some 300 different types of organisms" (144, 145), "up to 400 different phenotypes" (146, p. 165), to at least 400 to 500 different bacterial species (cf. 147, 148), and in addition some eukaryotic species, and at least one methanogenic archaeal species (cf. 149). For a listing of the main bacterial genera in human feces, see Tannock (150, p. 410S). On the other hand, the reported percentage of *E. coli* in the human gut has been steadily decreasing. Gordon (139) calls it "an extensive occupant of the intestine of man and the higher animals." Jacob (151) indicates that it is the predominant inhabitant (der häufigste Bewohner) of the gut. Clifton (152, p. 12; 153, p. 13) regards *Escherichia coli* as "the predominant organism in the intestinal tract of man." Finegold *et al.* (144) reported *E. coli* to be among the 25 most prevalent species in the fecal flora from humans fed a Japanese or a Western diet. "Human faeces usually contain 10^3 to 10^8 *Escherichia coli*/g, comprising approximately 1% of the total cultivable intestinal flora" (154). *Escherichia coli* is now accepted as but a minor ingredient of the intestinal flora in the adult human; numbers range between 0.2% and 1.5% of total cells (148). It has been regarded by Moore and Moore (155) as the 22nd most common fecal bacterial species, at 1.21%. They recognize 371 taxa, of which 261 are "new species yet to be named." Gerhardt and Iglewski (156) report one case of an individual who completely lacked or had very low numbers of coliforms in his stools. The problem of the disparity between the number of microscopically observable species and the number that could be grown in culture, already a concern of Escherich, has been partially overcome by the application of new methods for studying population dynamics in the intestinal tract such as quantitative polynucleotide chain reaction (Q-PCR) (157) and fluorescence *in situ* hybridization (FISH) (cf. 149). These techniques have changed perspectives on the prevalence of bacteria in many ecosystems related to the human colon. Thus "In 1995 ... the results of bacteriological culture ... [indicated] that obligate anaerobes outnumbered facultative anaerobes 1000:1. Since then, nucleic acid-based techniques have provided a new and more accurate perspective of this bacterial community. Nevertheless, a value of around 0.1% for *E. coli* holds true" (158). "Facultative species commonly described as 'enterics,' such as *Enterococcus faecalis* and *Escherichia coli*, are present at less than 0.1% of the total population" (146, p. 165). "As commensal organisms in our gut flora [*E. coli* is] a relatively minor component" (159). Numerous references can be found in a review by Paul (160). For some more publications on the extensive literature on this topic cf. Adam (161); Rotimi and Duerden (162); Kuhn *et al.* (163); Simon and Gorbach (164); Hall *et al.* (165); Yoshiota *et al.* (166); Roberfroid *et al.* (167); Adlerberth *et al.* (168); Adlerberth (169); Kirjavainen and Gibson (170); Tannock (171, 172); Schwiertz *et al.* (173); Tannock (174); Tuohy and McCartney (175). Escherich's own comments on the numerical incidence of *Bacterium coli commune* in the intestine are somewhat inconsistent. In one exhaustive review (88), the organism was said to constitute "only a vanishingly small fraction of the bacteria in the feces and in the intestinal contents" (p. 336), while later, in the same review, one reads about the "constant and rich occurrence of *Bact. coli com*. especially in the lower parts, in the empty intestinal canal, in the meconium and in diarrheal feces" (p. 417).

Four further points must be mentioned: (i) The influence of diet on the intestinal microflora, very much a concern of Escherich, who stressed the importance of mother's milk in early nutrition, remains a subject of close attention (144; cf. 145, 176, 177). (ii) It has become known that "The composition and functioning of [the intestinal] microflora plays an important role in the protection of the host against several pathogenic conditions such as colonic cancer, gastroenteritis, immunological disorders and developmental atopy" (cf. 149, 171, 172, 178, p. 42, and references therein; for an early reference, cf. 137). (iii) In spite of the recognized low numbers of *E. coli* in the gut, the mutagenicity of this organism and its occurrence or invasion of other tissues make it a prevalent and important pathogen (cf. 179). (iv) Escherich's pioneering studies on the prevalence of *Bacterium coli commune* in infants have been extended. For example, a paper by Bettelheim and Lennox-King, almost 100 years later (180), discusses the influence of the newborn's environment, such as the type of birth and, as main vector, the contaminated hands and uniforms of nursing staff [conclusions that forcefully remind one of the famous Semmelweis observations on the transmission of puerperal fever by physicians attending at childbirth (cf. 181, pp. 294–297)]. In a paper from Graz, one of Escherich's associates, a Dr. Robert Eberle, reports on the counting of infants' intestinal bacteria. He finds that only between 4.5% and 10.6% of the stainable bacteria will grow on his nutrient media. He discusses the possibility that this low ratio may be due not only to inadequate nutrients, but also to the possibility that many of the fecal bacterial types may be dead or weakened (182).

Escherichia: Vagaries of a Name

The name *Escherichia* is now so strongly associated with the intestinal bacterium first denoted by Theodor Escherich as *Bacterium coli commune* that the rather turbulent history of this name is often forgotten. The name, *Escherichia coli*, along with many other bacterial names, apparently was proposed in 1918 by Aldo Castellani and Albert J. Chalmers. Castellani, an eminent Italian physician, was at the time Lecturer at the London School of Tropical Medicine, and Chalmers was Director, Welcome Tropical Research Laboratories, Khartoum, Sudan. The name and the 1918 date are found in a 1919 and a 1920 Castellani and Chalmers publication: (i) the third edition (183) of their impressive "Manual of Tropical Medicine," p. 941, and (ii) Annales de l'lnstitut Pasteur (184), near the end of a 21-page paper "Sur la Classification de Certains Groupes de Bacilles Aérobies de l'Intestin Humain." The early "Escherichia" appellations are highly confusing. In the index to the "Manual" there are indeed a few references to *Escherichia coli* and extensive references to *Bacillus coli commune*, but none of these overlap. Similarly, in the "Annales" there is one mention of *Escherichia coli* Escherich 1886 (p. 619), and the organism is twice referred to as *B. coli* (pp. 610, 620). A look at the first edition (1923) of "Bergey's Manual of Determinative Microbiology" shows that the name, "*Escherichia*," had caught on like wildfire, for no less than 22 apparently different types of bacteria were designated as *Escherichia* (185, pp. 194–205). The second edition of Bergey (186) already indicated the rather uncertain or tentative nature of at least some of these assignments, for by then, although there were again 22 different species of bacteria classified as *Escherichia*, some of the earlier ones had been eliminated and others had taken their place (186, pp. 216–227). The third edition (1930) listed 29 species of *Escherichia* (187, pp. 316–331); the fifth edition of Bergey (188) had extensive revisions and has *Eschericheae* under Enterobacteriaceae as Tribe I, Genus I (188, pp. 389–396). At this point there were but two species under this genus, *Escherichia coli* and *Escherichia freundii*, and three variants of *E. coli* (*acidilactici, neapolitana*, and *communior*). Topley and Wilson (189, pp. 521–526) had the same three variants of *E. coli*. The appendix to the 1939 Bergey had 10 additional species according to Hauduroy *et al.* (190, pp. 226–232). Almost two closely printed pages in the 1939 Bergey listed bacteria either closely related to *Escherichia coli* or identical to it. In the sixth edition of Bergey (1948, p. 10), one reads: "One of the most unsatisfactory portions of recent classifications ... is the treatment given to organisms of the coliform-dysentery-typhoid group." The applicability of the term *Bacterium* to this group of bacteria was described (191, pp. 444–453). The seventh edition of Bergey (1957) had four species (*coli, aurescens, freundii, intermedia*) and four variants (the ones from the fifth edition plus *Escherichia coli* var. *communis*) (192, pp. 334–341). Almost a whole page was given to serology. "Within recent years there has been a great increase in interest in the serology of *E. coli* due to the association of certain serotypes with severe outbreaks of infantile diarrhea .... While it is not yet clear how many different strains of *E. coli* may be involved in the etiology of infantile diarrhea, the following have been found repeatedly in association with the disease: 026:B6; 055:B5; 0111:B4; 0127:B8; and 0128:B12" (p. 337). By the time of the eighth edition, almost 20 years later (1974), the situation had changed again, this time drastically (193, pp. 290–293; 194, pp. 293–296). "*Escherichia* is now regarded as a genus with only one species in which there are several hundred different antigenic specificities; together these specificities produce by different combinations of the O, K and H antigens several thousand serotypes. Two species that were in the genus *Escherichia* in the seventh edition of The Manual will now be found in *Citrobacter*, while a fourth species, *E. aurescens*, is thought to be a pigmented form of *E. coli* and is denied specific status" (p. 292). By 2005, in the second edition of "Bergey's Manual of Systematic Bacteriology," 18 pages are devoted to the genus *Escherichia*. A few important facets may be mentioned: (i) Comparative studies with rDNA. These show a close phylogenetic relatedness between *E. coli, Salmonella* spp., and *Citrobacter freundii*. In addition, relationships to *Shigella* are pointed out. "With the exception of *S. boydii* serotype 13, the DNAs of *E. coli* and the four *Shigella* species show such a high degree of relatedness ... that these species should be considered as a single species" (195). The distinction between these bacteria prevails, however, for reasons of historical/medical precedent and to avoid confusion in the literature and with existing surveillance systems" (196, p. 607). (ii) O, K, H, and to some extent F antigens: "Subdivision of *E. coli* can be carried out in many ways, but serotyping remains one of the most useful ways to subdivide the species on a global basis" (196, p. 613). (iii) Pathogenicity in relation to virulence factors: "Most *E. coli* strains are nonpathogenic and reside harmlessly in the colon; however, certain serotypes or clones play an important role in both intestinal and extraintestinal diseases .... In hosts with compromised defenses, *E. coli* can also be an excellent opportunistic pathogen" (196, p. 613). The longest part of the review is devoted to the topic of *E. coli* in human intestinal diseases. The complexity of this field is brought home by the discussion of as many as eight "currently recognized categories of diarrheagenic *E. coli*" (cf. 196, 197, p. 613) such as, for example, enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC). In addition, it has been recognized that "*Shigella*, which still stands as a genus with four species ... in reality belongs to the extremely diverse species *Escherichia coli*" (198). The magisterial "Topley & Wilson's Microbiology and Microbial Infections" has extensive discussions of the serology of *E. coli* (cf. 199, 200). One cannot help recognizing, in relation to Theodor Escherich, how far one has had to travel in order to confirm his motivation for studying the bacterial basis of at least one of the causes of childhood dysentery. One also bears in mind the role of *E. coli* in extraintestinal infections (cf. 196, p. 620), for, as we saw, Escherich was the first to demonstrate the role of the bacterium in the pathogenesis of bladder infections. The 2005 review ends with brief discussions of "the five species" of *Escherichia* (*coli, blattae, fergusonii, hermannii, vulneris*). So an initial exuberance in assigning the name *Escherichia* to a variety of microorganisms was eventually replaced by the recognition of *E. coli* as the serologically most prolific microorganism. Escherich was celebrated quite a while before Castellani's appellation: not only does one find quite a few references to *Bacterium coli* (Escherich), a common practice in denoting the discoverer of a microorganism in parentheses after the name of the organism, but already in 1889 one sees "B. Escherichii Trev. (*Bacterium coli commune* Escherich)" on p. 15 of a 36-page classification book "I Generi e le Specie delle Batteriacee," by Count Vittore Trevisan di Saint-Léon (201). The death in 1911 of Theodor Escherich, well known and celebrated, very likely stimulated the wide acceptance of the term *Escherichia*. Furthermore, the proposal by Castellani and Chalmers to reclassify a host of bacterial species strongly contributed to the acceptance of this term. No reference to a 1918 publication by these two authors could be found, not even in the exhaustive bibliography found in the 2005 Bergey's Manual (cf. 202, p. 940). An Italian reference (203), which I have not seen in the original, might possibly refer to *Escherichia*. Aldo Castellani (1878–1971) had an extraordinary career (cf. 5, pp. 261–263; 94, pp. 410–411). He obtained his M.D. degree in Florence in 1899, trained in bacteriology in Bonn, in tropical medicine in London, went to Uganda and was the first, near the end of 1902, to show *Trypanosoma brucei* in the cerebrospinal fluid of a patient suffering from sleeping sickness, became Director of the Bacteriological Institute and Professor of Tropical Medicine at the Medical College of Colombo in Ceylon (Sri Lanka), discovered *Shigella sonnei* (he had called it *Bacillus ceylanensis*), wrote 200 mycological articles, discovered *Candida tropicalis, Candida pseudotropicalis, Candida guiliermondii*, and *Trichophyta rubrum*, was simultaneously Professor of Tropical Medicine at Tulane University (later at Louisiana State University), at the London School of Hygiene and Tropical Medicine, and at the University of Rome. He also practiced medicine at Harley Street in London (eventually became Sir Aldo Castellani), and spent his final years in Lisbon as Professor at the Tropical Disease Institute and personal physician to the exiled Queen of Italy. This is not all. His and Albert Chalmer's Manual of Tropical Medicine is an immense and highly impressive book (the first edition, 1910, has 1212 pages; the third edition, 1919, has 2436 pages) (183, 204). In 1959, with Frederick Reiss, he founded the International Society of Dermatology (initially the International Society of Tropical Dermatology) and was its first President. In England, his patients included the Maharaja of Mysore, Rudolf Valentino, and Giuglielmo Marconi. To cap all of this, perhaps, he was also at one time the personal physician of Benito Mussolini. Much of this is described in Castellani's fascinating autobiography (205). Not too many people know that we owe the term *Escherichia coli* to Mussolini's personal physician.

Monod, in his 1942 doctoral thesis (206), always uses the term *B. coli* (I counted 61 times in this fascinating work) without initially indicating the meaning of *"B."* and he never uses *Escherichia coli or E. coli*. An amusing episode, told by Lwoff about Monod's introduction to *E. coli*, deserves mention: "[I] advised him to use a bacterium able to grow in a synthetic medium, for example *Escherichia coli*. "Is it pathogenic?" asked Jacques. The answer being satisfactory, Monod began, in 1937, to play with *E. coli* and this was the origin of everything ..." (207; cf. 208, 209). See Cohen (210) for an admirable account of the contributions of work at the Paris Pasteur Institute to the development of molecular biology. As mentioned near the beginning of this essay, Watson in the various editions of his influential textbook, "The Molecular Biology of the Gene," uses *"E. coli"* throughout, and also does not follow bacteriological practice in initially using the whole designation. There is an amusing disparity in nomenclature in Escherich and Pfaundler's review of Escherich's bacterium: in the clinical part, written by Escherich, one consistently sees *Bacterium coli commune*, while in the physiological part of this highly impressive review, written by Pfaundler, one just as consistently sees *Bacterium coli*, and never *Bacterium coli commune*. Escherich, in a footnote to this review, indicated that the designation "commune" in *Bacterium coli commune* refers to the common occurrence of this organism in infants as well as in adults (88, p. 335). He eschewed the binomial system for naming his bacteria. He used *commune* to emphasize that it was present in all the specimens that he had tested, and that it was an obligate intestinal bacterium. Although he could find no correlation between the appearance of the bacteria and the symptoms and occurrence of childhood diarrhea, he proceeded, in true scientific and modern-sounding fashion, to continue his work. He proceeded to describe the appearance, the cultural characteristics, and the possible pathogenicity of his bacteria (Fig. 5).

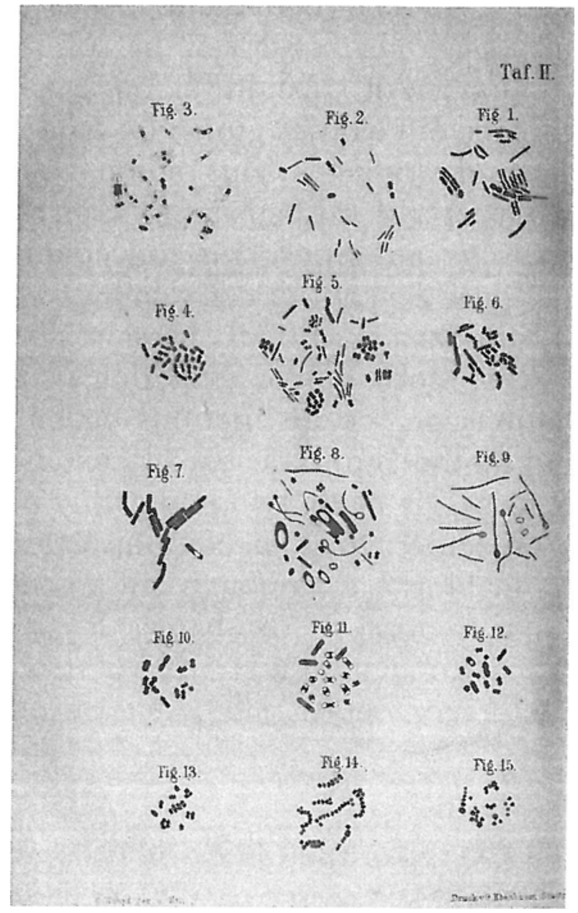


Figure 5 Photographs and some of the accompanying legends at the end of Escherich's 1886 book. *Fig. 4. Bacterium coli commune* from a 6-day-old potato culture; predominantly short, constricted (eingeschnürte) shapes. *Fig. 6. Bacterium coli commune* from an 8-day-old gelatin plate colony (Gelatineplattencolonie); readily apparent rod-type. *Fig. 7. Bacillus subtilis*; one bacillus at the stage of spore formation. *Fig. 10. Bacterium lactis aerogenes* from an 8-day-old gelatin test tube. All photographs, 970-fold magnified, were taken by Charles Workman, M.D. from Belfast, a visitor in the Bacteriological Laboratory of the Munich Pathological Institute, using isochromatic plates, and copied photographically (durch Lichtdruck vervielfältigt). Copyright 2006, Elsevier, Inc. http://www.sciencedirect.com/science/article/pii/S0065216406600051 doi:10.1128/ecosalplus.ESP-0025-2013.f5

ESCHERICH'S NEGLECT OF HIS DISCOVERY OF *CAMPYLOBACTER JEJUNI* AND OF SOME OTHER BACTERIA

Escherich is celebrated as the undoubted discoverer of what is now known as *Escherichia coli*. He also, and at the same time, discovered what he called *Bacterium lactis aerogenes*. In addition, he is almost certainly the discoverer of yet another organism, *Campylobacter jejuni*, responsible for more cases of diarrhea than those brought about by pathogenic strains of *E. coli*. The story is intriguing and again shows Escherich as a superb bacteriologist and clinician. As we saw, he spent just 2 weeks in Naples during the 1884 cholera epidemic. Scarcely a month later, at the 3 December 1884 session of the Munich Medical Society, he presented a long report on his experiences. In the course of his discussion on observations of Koch's "commabacteria" in cholera feces, he mentioned that he frequently found "tooth spirochetes" as well as several other bent forms in these feces. Almost as an aside he stated that, several weeks earlier, i.e., presumably before he went to Naples, he found a bent bacterium in the intestine of a 4-day-old baby, and that this organism was decidedly different from "real" commabacteria (41). One can say that his interest in this bacterium was stimulated by a comparison with the cholera bacterium. He observed this organism, therefore, before his discovery of *Bacterium coli commune*. A much more detailed report on the bacteriology of a "bent" organism appeared in two reports from 1886, one on what he called *Vibrio felinus* obtained from the feces and intestine of a diarrheic cat (211), the other, with illustrations, from the intestinal canal and the feces of infants (212). The latter organisms differed from the dental spirochaetes. To study the possible relation between the vibrios and various intestinal disturbances, he observed samples from 72 sick infants under the microscope and found the vibrios in only 41 cases. As a careful clinician, he shied away from concluding that these vibrios were a cause of infant diarrhea, but he indicated that their presence could not contradict a serious prognosis. He was far more conservative a year later, near the end of a historical review on intestinal bacteria and the etiology of intestinal diseases. He stated that there was no relation between the vibrios and the etiology of intestinal diseases (122). This review is of interest to readers in the United States, for with his customary thoroughness Escherich stated that American physicians were the first to observe the hot summer months to be associated with the enormous, almost epidemic, increase in mortality of infants up to the age of 1 year. It was called "summer complaint" or "summer cholera," and was regarded until the middle 1800s as "an entirely American disease." Escherich indicated that the first detailed descriptions were given by Benjamin Rush in his 1789 "Medical Inquiries and Observations." Kist (213) has suggested that Escherich did not pursue the study of his vibrios since he was not able to grow them in culture. Kist (213), exactly 100 years after Escherich's detailed description, resuscitated Escherich's essentially forgotten discovery of *Campylobacter jejuni* (not mentioned in any of his obituaries). The end of Kist's paper is worth quoting: "Besides the discovery of *Escherichia coli* it has now become highly probable to add the first description of *Campylobacter* to Escherich's scientific accomplishments, although he never fully recognized its actual significance." Only in the 1970s was *Campylobacter jejuni* recognized as an emerging human pathogen, the leading cause of enteritis and enterocolitis (214; cf. 215, 216, 217).

There is another matter of a near miss, that of the dysentery bacillus. This is brought out most clearly in Béla Schick's admirable reminiscences of Escherich, written some 46 years after his teacher's death: "Escherich's preoccupation with the colon bacillus stood in his way of making another important discovery. When he cultured colon bacilli from the gastrointestinal tract, he focused his attention on the gram-negative strains which produced gas in the agar-culture medium and discarded those strains which did not produce gas. This discarded bacillus was the dysentery bacillus later described by Flexner" (4, p. 115).

EXTENSIVE STUDIES WITH *E. COLI*, LONG BEFORE THE ADVENT OF MOLECULAR BIOLOGY

Early Interests

Escherichia coli is often regarded to have been an obscure organism lifted from neglect by the rise of molecular biology. As Lord Byron would have said, it was held to have awoken one morning and to have found itself famous. Thus, for example, one reads in Greame Hunter's book, "Vital Forces, the Discovery of the Molecular Basis of Life" (218, p. 314), "Arguably, the main achievement of the Phage Group was to introduce the bacterium *Escherichia coli* into biochemistry." Similarly, in Franklin Harold's book, "The Way of the Cell," p. 65, one reads "Project *E. coli* grew out of the researches of André Lwoff, François Jacob and Jacques Monod, initiated in Paris just before the Second World War" (219). Hobom (113), in an article on the occasion of the centenary of the discovery of *E. coli*, calls this inconspicuous intestinal inhabitant the "laboratory mouse of the molecular biologists," and states that "the history of *E. coli* is mainly also the history of molecular biology." This is decidedly not so. *E. coli* was famous right from the beginning. It almost immediately attracted the attention of bacteriologists, physicians, biochemists. As early as 1894, that is, just about 9 years after Escherich's description of the organism, a textbook published in the United States stated: "The fact that it is always with us in most intimate association with certain of our life processes, together with the fact that it is known to appear in organs other than that in which it is normally located, and that its occurrence in diseased conditions is not rare, justifies the opinion that it is one of the most important of the micro-organisms with which we have to deal" (220, pp. 305–306). Familiarity with *Bacterium coli commune* rapidly spread. Thus, some 3 years later one reads: "few organisms are more frequently met in the every day work of a bacteriologist than *Bacterium coli commune*"

(139, p. 438). An impressive testimony to the early and extensive interest in the organism is provided by the 141-page review, simply called *Bacterium coli commune*, published in 1903 by Escherich and Pfaundler, that is, just under 20 years after the discovery of the organism, in volume 2 of the highly regarded "Handbook of Pathogenic Microorganisms." The cumulative effect of this article is one of concentrated, hard work, elegance, and critical familiarity with the latest contributions to an exploding field of medicine and bacteriology. The inclusion of an article on *Bacterium coli commune* in a "Handbook of Pathogenic Microorganisms" is a recognition of the pathogenicity of this organism. The review has 20 pages of references, with about 30 citations per page, many, but by no means all, dealing with *Bacterium coli commune*. The article is a useful milestone for then current bacteriological techniques and approaches, as well as a critical evaluation of the clinical and pathological ramifications occasioned by this organism. The first part of the review, some 88 pages written by Pfaundler, describes the "Morphology and Biology" of the organism; the remaining two parts, written by Escherich, deal in a short section with "*Bacterium coli commune* under physiological and pathological conditions" and, in a long section, with "The Bacteria of the Coli-Group as Causative Agents of Disease." The details do not concern us here, except for the recognition that this organism elicited diversified interest, bacteriological and pathological, right from the start. We read about the morphology of the organism under different conditions of growth, on its fermentative behavior, its formation of lactic and other acids, of indole, skatole, methyl mercaptan, ammonia, hydrogen sulfide, on ways of distinguishing it from *Bacterium typhi*, on its growth under anaerobic versus aerobic conditions, antagonism to growth of other bacteria as a possible role in controlling the variety of intestinal bacteria, on the effects of desiccation, of pressure, and so on. Chemical criteria for distinguishing between different bacteria were probably first used to distinguish between *Bacterium coli* and what Escherich and Pfaundler (88, p. 383) call "Typhuskultur." Thus, while *B. coli* converts glucose to D-lactic acid, *Bacterium typhi* converts it to L-lactic acid (221). A 5% lactose solution is rapidly fermented by *B. coli*, but not by *B. typhi*. A long section is devoted to its pathogenicity in animal experiments and to its occurrence outside the intestinal tract. No firm evidence is presented for the increase in incidence of *Bacillus coli commune* in dysentery and a possible role of this organism as a causative agent of this disease. W. Kruse is cited as the discoverer (1900) of the dysentery bacillus. Escherich is extremely critical about accepting various studies indicating changes of *Bacterium colon commune* populations as associated with pathogenicity in humans. On the other hand, he accepts reports on the occurrence of a fatal intestinal colisepsis in a number of babies. The possibility that this organism may cause peritonitis and diseases of the gall bladder is also discussed. Far more credence is given in a lengthy section (7 pages) to the observations that this organism is the most common cause of diseases of the urinary tract. The possible role of the organism in a large number of other diseases is discussed. The article ends with the statement: "One cannot doubt the existence of independent coli effects, although the clinical picture and the type of these diseases have to be established by further research." Although Escherich was the first to demonstrate and to stress the marked differences in the pathogenicity of his organism to animals as contrasted to humans, the various pathogenic strains that later were discovered were not known in his time. However, Ludwig Brieger had claimed as early as 1895 that there were resemblances between poisons due to *Bacterium coli* and to the organism causing typhus (222).

The Development of Classical Biochemistry Depended Heavily on Studies with *Bacterium coli commune*

What is of greater interest to contemporary readers is the recognition that work with *Bacterium coli*, later called *Escherichia coli*, led to an impressive variety of fundamental biochemical discoveries, long before the biological insights that initiated and advanced molecular biology. A bedrock of biochemical insights was gained by work with this organism. In the words of Joshua Lederberg: "During the first half of the twentieth century, *E. coli* was probably the single most studied bacterial species for basic physiological and metabolic investigation, but it was rarely mentioned in general biology texts" (223). Textbooks of biochemistry by and large tend to minimize or to ignore the sources of their substances or extracts of interest, but in textbooks of bacteriology one finds clear correlations between observations and the bacteria or the bacterial material with which they were made. Two of the standard textbooks of bacteriology, one current in the early 1930s (224, 225), the other current in the 1930s and 1940s (226, 227, 228) were consulted. The "Index to Microörganisms" in Buchanan and Fulmer's scholarly work has far more references to *Bacterium coli* than to any other microorganism. This organism again predominates in the Index of all three editions of Marjory

Stephenson's influential treatise. Thus, *B. coli* in the Index to the first edition occupies an entire page (p. 312), with more entries than any other microorganism or indeed any other topic. In the Index to the 1939 edition (p. 380), *Bacterium coli* again has more entries than any other subject; here for the first time one sees *Escherichia coli*, with the cursory remark "see *Bacterium coli*." The organism again predominates in the Index for the 1949 edition with all entries (p. 389) under "*Escherichia coli* (*B. coli*)" and none under *Bacterium coli*.

We start this survey of pre-molecular biology *E. coli* discoveries with a quotation from Juda Quastel, a major 1920s contributor. The high esteem in which J. H. Quastel was held, not only for his pioneering work with *E. coli*, was shown by the fact that he and C. S. Hanes, an eminent enzyme kineticist, served as honorary presidents of the XIth International Congress of Biochemistry, Toronto, 1979. The author remembers an outdoor reception at that Congress, with Hanes and a smiling Juda Quastel, resplendent in a spotless off-white suit, shaking hands with a line of attendees. He started his work with *Bacillus pyocyaneus* (*Pseudomonas aeruginosa*), but soon abandoned this organism since it tended to form sticky, mucilaginous growth. "I ... eventually concentrated on *Bacillus coli* (or *Escherichia coli*) as a very suitable organism, easily grown and washed, and capable of forming saline suspensions that could be handled with great accuracy. … Our systematic work with *E. coli* launched this organism into the field of biochemical research. Today it is one of the most popular organisms of investigation in the field of molecular biology" (229, pp. 72–73). Experiments with *E. coli* suspensions, performed at the Biochemical Laboratory of Cambridge University, resulted in the recognition of "at least 56" specific dehydrogenases (229, p. 73), leading directly to the fundamentally important idea of "active centers" (230, 231, 232, cf. 233, 234) and grew into a series of highly important insights into the nature of enzymatic action. The idea of what is now called the enzyme active site was advanced by Quastel, following work with *Bact. coli*, in the very year that Sumner with his studies on urease demonstrated the protein nature of enzymes. Quastel's proposal of an active center was, however, independent of the actual chemical nature of enzymes (cf. 235, p. 394).

Further, Quastel's interest in the dehydrogenation of succinate to fumarate led directly to his discovery of the phenomenon of competitive inhibition, exemplified by the effect of malonate on succinic dehydrogenase (236, 237, 238). This action of malonate led workers in Szent-Györgyi's laboratory to demonstrate the catalytic role of fumarate in cellular respiration (239, p. 33). Furthermore, the phenomena of malonate inhibition and of the role of fumarate played a central role in Krebs' discovery of the citric acid cycle (240). It is not often realized that it was work with *E. coli* that led to these fundamentally important biochemical insights (cf. 241, p. 154). Work by others in Cambridge with *Bacterium coli communis* led to further basic discoveries. Thus, Barnet Woolf's classical work with *Bacterium coli communis* (242, 243) first advanced what he modestly called "a limited hypothesis on enzyme action" (242, p. 482) later known as the ternary-complex mechanism, a bedrock of understanding of the mechanism of a host of enzymatic reactions (cf. 244). J. B. S. Haldane in his classic 1930 book "Enzymes" has extensive references to "*Bacillus coli*," far more in fact than to any other bacterium, and he devotes a number of pages to what the calls "Quastel's Theory of Dehydrogenases," based entirely on work with this organism (245, pp. 183–185).

A few more discoveries made with *Bacterium coli communis* bring home its importance in the development of biochemistry, long before the advent of molecular biology. Work with *B. coli* led to the term colicin, coined by André Gratia in 1925. *B. coli* was one of the bacteria in which the enzyme hydrogenase was first demonstrated (246). Leonard Hubert Stickland, in his very first paper (247) on what became known as the Stickland reaction, referred to similarities between his observations with *Clostridium sporogenes* and "certain anaerobic energy-yielding reactions of *Bact. coli* (248)."

A mere 2 years after Eduard Buchner's revolutionary discovery in 1897 of cell-free alcoholic fermentation by yeast extract (cf. 249), Arthur Harden began his classical studies on the nature of alcoholic fermentation not, as may be assumed, with yeast preparations but with *B. coli* (250, 251). His interest in fermentation, stimulated by work with *B. coli*, led to his classical studies on alcoholic fermentation by yeast for which he received an early Chemistry Nobel Prize. The first recognition of chemical relationships between fermentation products and fermented substrates were credited to the "theoretical speculations" that Arthur Harden (251) brought to bear on his studies with *B. coli* (cf. 252). Although he soon switched to yeast (253, 254), his interest in *B. coli* as a model system remained for many years (255). Kluyver in his 1931 book "The Chemical Activities of Microorganisms" gives extensive data on the fermentation of glucose by *B. coli* (256, pp. 56–57). *B. coli* was also one of the predominant organisms used in varied and extensive studies on amino acid metabolism. Umbarger states in one of his impressive reviews of amino acid biosynthesis and its regulation: "The material is heavily weighted toward the regulation of amino acid biosynthesis in *Escherichia coli* and *Salmonella typhimurium*. This bias could not be avoided, since these organisms have attracted most of the workers in the field" (257, p. 534). One of the highlights was the isolation of shikimic acid from *Escherichia coli* mutants (258).

Bacterial Genetics Originates with *Bacterium coli mutabile*

B. coli communis played a basic role in the development of bacterial genetics. In the earliest years, there was a somewhat perfunctory interest in the possibility that different strains of *B. coli commune* existed. Thus in 1886, just a year after the discovery of the organism, Escherich states in his notable book: "In an earlier section I indicated that I was more interested in the description of closely related types occurring in feces than in their detailed differentiation. I referred particularly to the large group of colon bacteria that in my opinion share enough properties to justify being summarized under one designation. Individual observations point to the possibility that more precise investigations and perfected methods will lead to the differentiation of further species." Some 13 years later, when Escherich was in Graz, Dr. Henry Lee Smith, a visitor from Baltimore, was asked to look into the possibility of different *Bact. coli* strains. He compared morphological and biological characteristics of the organism obtained from the feces of different children and concluded that there were distinct differences in *B. coli* obtained from the feces of different children, particularly in the lack of agglutinating cross-reaction by the serum of a guinea pig that had been immunized against one of the strains. He concluded that the differences were not dependent on nutrition, since, at least in one case, there was no change in serological properties in the course of the child's transition from mother's milk to artificial food (259). A year earlier A. Péré (260) had shown that reported differences in fermentative properties of *B. coli commune* could be traced to different sources of the organism. The 1903 review by Escherich and Pfaundler has many pages of references and discussions of variations in *B. coli*. There was no consensus as to the meaning of such variations. On the one hand, some changes were reversed by simple further cultivations, and on the other hand, uninfluenced (unbeeinflusste) changes in some strains were reflected by stable morphological, fermentative, and nutritional properties. These latter strains were designated as different types (Spielarten) or variations (88, p. 405). There was no discussion of the permanence or the inheritance of these traits.

Max Neisser in a report on the dissertation of his student Rudolf Massini used the term *Bacillus coli mutabilis*, for "a case of mutation according to de Vries in bacteria" (261). A year later Massini's work was published in a 40-page article on what he called *Bacterium coli mutabile* (262), with a clearer statement on his work's genetic significance: "This work constitutes a contribution from bacteriology to the theory of mutation, as has been carried out by Hugo de Vries in the field of botany ... the mutation is inherited as soon as it has occurred ... it seems permissible to use de Vries's theory in bacteriology, especially in our case" (262, pp. 289–290). This work was done in the Institute of Experimental Therapy in Frankfurt whose director was none less than Paul Ehrlich. The bacteriologist Max Neisser, head of the bacteriology section of Ehrlich's institute (cf. 263), is not to be confused with the physician Albert Neisser of *Neisseria* fame. Burri and Düggeli (264, p. 174) also spoke of mutations in coli stems. The idea of bacterial mutations faced criticisms. In a later paper, Burri (265) strongly attacked Massini's conclusions and claims that one "did not deal with a mutation in the sense of DeVries but with a phenomenon of adaptation." "Although later workers concluded that Burri's culture was apparently not *E. coli mutabile*, Massini's work was largely discounted because of Burri's experiments" (119, p. 145). The lasting inheritance of new traits in typhus, paratyphus, and related bacteria was vigorously defended by Reiner Müller (266, 267) and especially by F. H. Stewart (268) for the "paracolon mutabile colon group." The latter paper began with the statement: "Mendel's principles of variation, formed from the study of the genetics of higher plants and animals, can be applied also to bacteria, and that acquired virulence in bacteria is the result of Mendelian variation." He continued, quoting Neisser and Massini, by stating, "This thesis is not entirely new."

Some detail on Massini's work may be of interest, exactly 100 years later, for it was impressively "modern." Massini's *Bacterium coli mutabile*, in contrast to his initial *B. coli* strains, was able to ferment lactose. The lactose-fermenting ability of this strain was observed by use of so-called Endo agar (269), which contains lactose, and fuchsin as an indicator of acid formation. After a few days a bacterium able to ferment this sugar produced dark red papillae on this agar, whereas the bacterium incapable of such a fermentation produced a white colony. The papillae, upon replating, grew as red-colored bacteria. Massini concluded that, during the growth of the colony of initially lactose-negative bacteria, a sudden change to lactose-positive had occurred, without intermediate forms. The bacterial subclones retained the capacity for lactose fermentation; that is, one had obtained lactose utilizing *mutations*. Massini advanced numerous experimental criteria to ascertain that he had always started with single colonies of "white" bacteria, that is, that no "red" colonies were present from the start. In Massini's day, the genetic nature of these mutations could not be proven conclusively. It is not clear from a reading of Massini's and later papers whether the term *B. coli mutabile* referred to the strain before or after mutation. Thus, Arnold Burk, just a year later, confirmed Massini's results, but argued that the mutation resulted in an organism indistinguishable from *B. coli* (270). He held that the original strain was related to *B. coli*, but should not be regarded as this organism. In fact Escherich and Pfaundler's 1903 review (cf. "Early Interests") made the point that *B. coli*, in contrast to *B. typhi*, was a lactose fermenter. Here one had a criterion for the important distinction between *B. coli* and *B. typhi*. Clearly the earlier work on *B. coli* may have dealt with a strain different from Massini's or with an unrecognized mutant, but this in no way invalidates Massini's conclusions. I. M. Lewis, almost 30 years later, stated that "The subject of bacterial variation and heredity has reached an almost hopeless state of confusion" (271), but he obtained the valuable measurement that the frequency of the change from *E. coli* to *E. coli mutabile*, that is, from lactose-negative → lactose-positive, was in the order of 1 per 100,000 viable cells. He took care to use the word *variation*, and not *mutation*. After a further ten years, a summary of Massini's paper in the authoritative Topley and Wilson's textbook, third edition, also avoided the terms *mutant* or *mutation*: here *Bacterium* (not yet *Escherichia*!) *coli mutabile* was called "an interesting type of variability" and a "curious organism" (272, pp. 297, p. 677). At the time no less a person than André Lwoff (273, p. 146) still referred to *Escherichia coli mutabile*. By then the assumption, implicit in many of the early papers in the field, that it was the nutritional environment that brought about bacterial mutations, began to be replaced by the recognition that a given nutritional environment, rather than initiating mutations, simply allowed preexisiting mutants to grow. As noted by Schlegel (49, p. 136), neither Massini nor Lewis recognized that their data showed the spontaneous nature of their mutations. As an illustration of the recalcitrance of habit over the elusiveness of epochal conclusions, Lewis for instance left his area of investigation and devoted himself to bacterial cytology (cf. 49, p. 136). It bears remarking, in an article on *Escherichia coli*, that it was work with *E. coli*, infected with bacteriophage, that initiated the replacement of a Lamarckian by a Darwinian process of selection in bacterial selection (274). Massini and Arnold Burk and Burri and Lewis, and others not cited here, have lapsed into near obscurity, while Luria and Delbrück received a Nobel Prize. From that point, one can say, bacterial genetics came into its own.

SUMMARY

Theodor Escherich's success in discovering *Bacterium coli commune* stemmed from a mixture of luck and of the limitations in bacteriological techniques available at the time. Luck came in three forms: the preponderance of this organism in the stools of infants in contrast to adults, the obligate, rather than what he called facultative, presence of this organism in all the stools that he examined, and above all the remarkable ease with which it could be cultivated. More sophisticated techniques for bacterial cultivation, made available long after Escherich's time, would have demonstrated that the stools are populated by as many as 400 to 500 different kinds of microorganisms, a recognition that might well have relegated *Bacterium coli commune* to a subsidiary position in the pantheon of fecal exuberance. One should probably add technical limitations, when they help to contribute to a discovery, to the confluence of lucky circumstances, since later technical advances or changes cannot possibly be used to deprecate earlier standards of achievement. This recognition applies particularly to the spectacular achievements in bacteriological discovery, due especially to the new techniques of bacterial cultivation introduced mainly by Robert Koch. The very inability to detect more discriminatory organisms, not cultured with the media known at the time, strongly helped in the discovery of *Bacterium coli commune*.

The discovery *of Escherichia coli* in 1885 occurred at a time that saw the confluence of a number of factors. This discovery occurred during the so-called golden age of bacteriology when it became abundantly clear that many

diseases were caused by newly discovered pathogenic bacteria. As examples, tuberculosis was shown to be due to an infectious bacterium, and cholera was shown to be due to the so-called comma bacillus. These various discoveries were brought about by a refinement in bacteriological techniques and by the promulgation of Robert Koch's postulates of infection (actually first advanced by Koch's teacher Jacob Henle, cf. 275, p. 449). The isolation of pathogenic bacteria, and the elaboration of criteria to establish their causal relationship to a number of devastating diseases, at long last demolished still prevalent ideas of disease etiology that pitted the traditional miasmatists against the upstart contagionists. (See 118, for a discussion of these two views, their relationships, and their applications in the service of preventive medicine.) Theodor Escherich, a highly versatile pediatrician skilled in bacteriological techniques, was very much aware of the devastating toll that childhood dysentery took upon early human lives. More and more convinced that dysentery, in analogy to the cholera epidemic he had studied in Naples, was also of bacterial origin, he was determined to establish this postulate by finding the causative organism. He failed in this effort, as he himself acknowledged. The welter of microorganisms in the human intestine made it very difficult to determine which of these, if any, might be the culprit, since they all occurred in normal as well as in diseased intestines.

Escherich's work was helped by three factors. First, he had learned Robert Koch's brand-new bacteriological techniques from Wilhelm Frobenius, who had learned them in turn from Robert Koch himself. Second, Escherich's careful bacteriological researches on the feces of very young children showed that one of these bacteria, which he called *Bacterium coli commune*, predominated at some early stages of life. Third, this organism claimed attention by its incredible ease in culturing. Its apparent prevalence and its ready growth prompted Escherich and many of his contemporaries to select this organism for further study. An additional advantage, apparent early but expanded by later work, was that this readily cultured organism manifested a highly impressive variety of strains. It became the first bacterium, in the form of the so-called *Bacterium coli mutabile*, to which genetic theories were applied. Thus, studies by Rudolf Massini in 1906 initiated the field of bacterial genetics. In addition, long before the advent of molecular biology with *E. coli* as its flagship organism, *Bacterium coli commune* was a favorite model organism in many basic biochemical studies. Investigations with this organism suggested the notion of the active site of enzymes; work with a large number of dehydrogenases from this organism led to the discovery of competitive enzyme inhibition. Studies with enzymes from this organism suggested the so-called ternary complex mechanism, a standard enzyme mechanism, led to the discovery of hydrogenase, initiated studies on alcoholic fermentation. It was, along with *Salmonella typhimurium*, a favorite organism for the study of amino acid metabolism. Clearly *E. coli* had a venerable history before its adaptation as the organism of choice in the development of molecular biology, a choice that continues to this day and that shows no signs of abating. By now the "coli" in *E. coli* is but a distant echo of its source, as irrelevant to most as is *"E.,"* intended originally in celebration, but by now, to adopt Thomas Carlyle's quotation at the top of this essay, but maundering and mumbling.

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