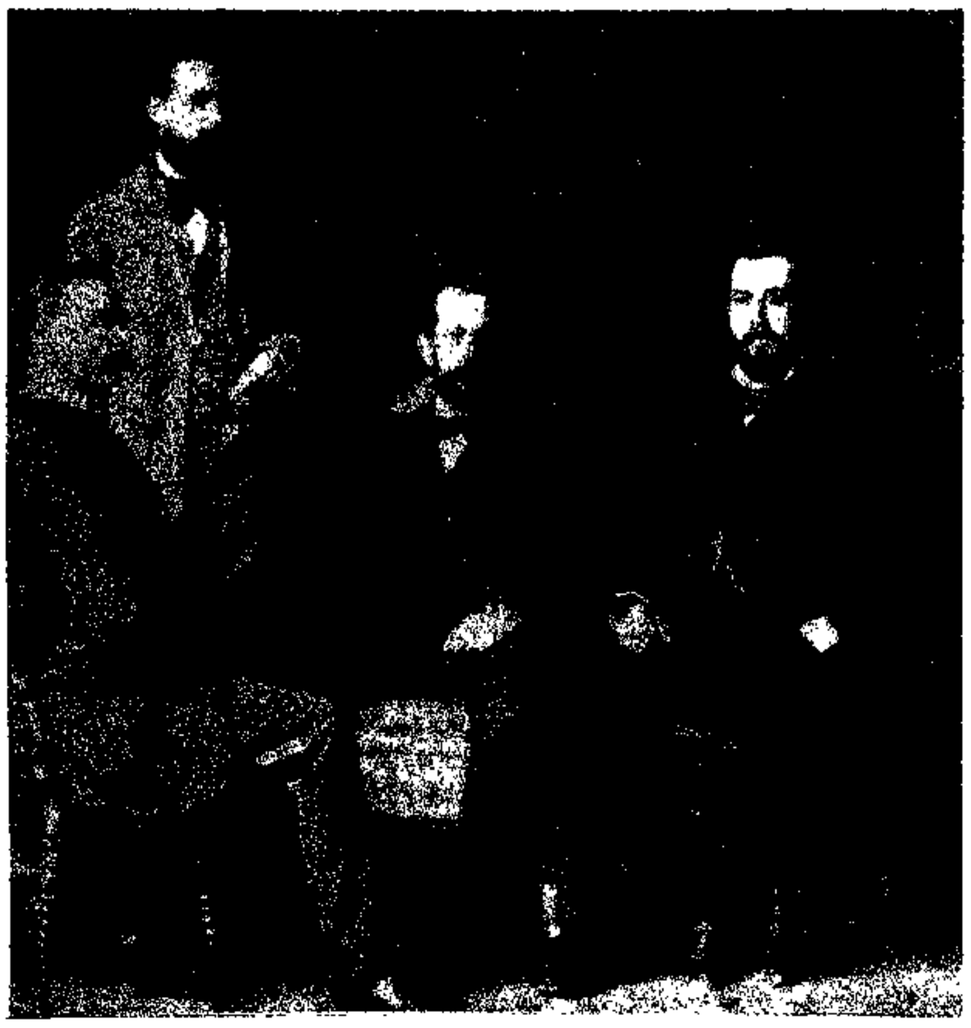
Special Issue on S. N. De and
Cholera Enterotoxin

Historical Note: Robert Koch, Calcutta and the Comma Bacillus

P. Balaram

The origins of cholera are shrouded in antiquity, but Calcutta has long been not only its home but also the setting for some of the most dramatic advances in understanding the causes and nature of the disease. While S. N. De's discovery of the cholera enterotoxin is a landmark of the field in recent years, it was here, over a century ago, that Robert Koch (1843–1910) isolated and described the causal agent of cholera – the comma shaped bacillus *Vibrio cholerae*[1].

Koch, undisputably the founder of modern bacteriology arrived in Calcutta on 11 December 1883, on his 40th birthday as head of the German Cholera Expedition. Spurred by the outbreak of cholera in Egypt in 1883, both France and Germany set up cholera Commissions – the former under Louis Pasteur (1822–1895) and the latter under Robert Koch. The germ theory of disease had been established by this time, largely due to the work of Pasteur and Koch, and it was hoped that cholera might now be understood and controlled. Pasteur was aging by this time and in his absence the French *Mission Pasteur* was led by Isidore Straus (1845–1896) and included Pasteur's young assistant Louis Thuillier (1856–1883). Both French and German expeditions were in Egypt in 1883, where Thuillier was to die of cholera, with Koch attending his funeral. The ill-fated French team turned back, while Koch moved on to Calcutta, where success came quickly.



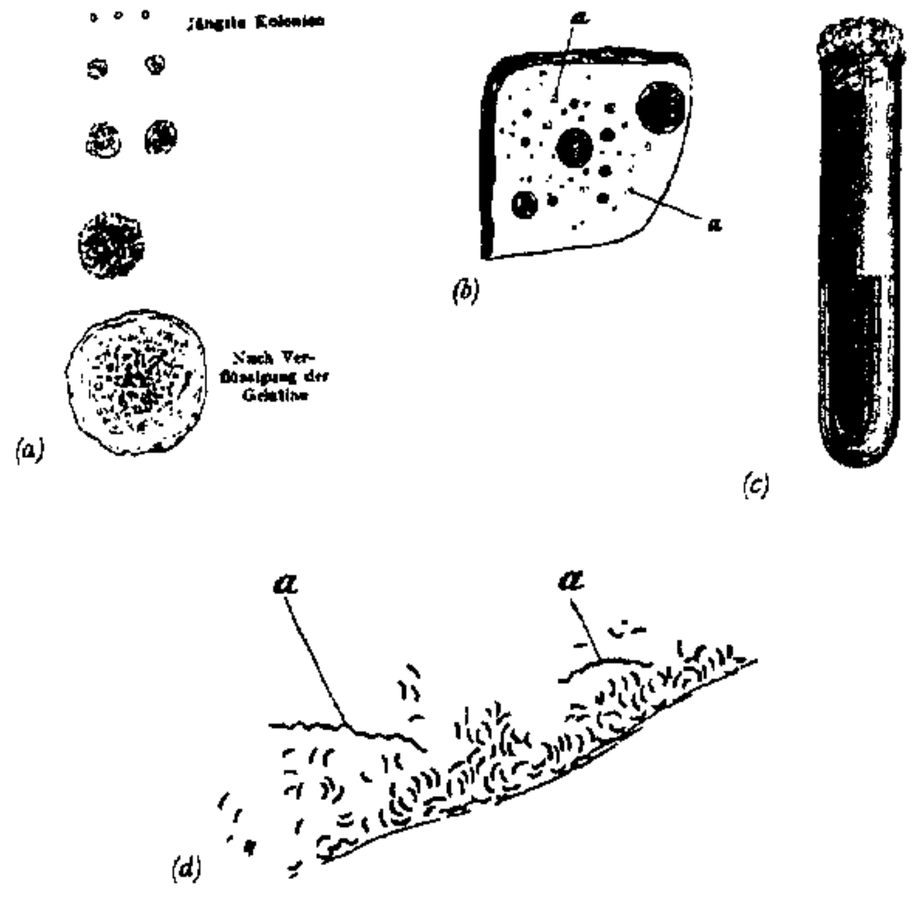
Members of the German Cholera Expedition of 1883–84. From the left: Gaffky, Treskow (standing), Koch, Fischer. Taken from 'The Cholera Expedition.'

Koch's major advantage in Calcutta was the availability of fresh autopsy samples to obtain a pure culture of the cholera organism. Working with autopsy samples from a 22 year old patient at the Sealdah hospital, Koch confidently proclaimed the isolation of the "comma shaped bacillus" as the causative agent of cholera. In Koch's words[2]:

> "It can now be taken as conclusive that the bacillus found in the intestine of cholera patients is indeed the cholera pathogen.... We have determined special properties of the bacillus that make it possible to definitively separate the cholera bacillus from other bacteria. These characteristics are the following: The bacillus is not a straight rod, but rather is a little bent, resembling a comma. The bending can be so great that the little rods almost resemble half-circles. In pure culture these bent rods may even be S-shaped... They are very actively motile, a property which can best be seen when examining a drop of a liquid culture attached to a cover slip... Another important characteristic is the behavior of the bacteria in nutrient gelatin. Colonies are formed which at first appear compact but gradually spread out as the gelatin is liquefied. In gelatin cultures,

[1] This account of Koch's involvement in cholera research is based on the relevant sections of the outstanding biography, "Robert Koch: A Life in Medicine and Bacteriology" by Thomas D. Brock, Science Tech Publishers, Madison, Wisconsin and Springer-Verlag, Berlin 1988.
[2] Quotation from the reference cited in footnote 1.

colonies of the cholera bacillus can therefore be readily distinguished from colonies of other bacteria, making isolation into pure culture easy... Up to now, 22 cholera victims and 17 cholera patients have been examined in Calcutta, with the help of both the microscope and gelatin cultures. In all cases the comma bacillus



Pure cultures of the cholera vibrio. (a) Koch's drawings of colonies on a nutrient gelatin plate. (b) Lower magnification, showing characteristic colonies (labeled a). (c) Pure culture growing in nutrient gelatin stab. (d) Cover glass preparation of a pure culture, showing the long screw-shaped filaments that sometimes developed (labeled a). Taken from 'The Cholera Expedition'.

and only the comma bacillus has been found. These results, taken together with those obtained in Egypt, prove that we have found the pathogen responsible for cholera."

After some initial scepticism, Koch's conclusions on the etiology of cholera were accepted at the Berlin conference of 1884. Although Koch did not take cultures of the cholera bacillus back to Europe from Calcutta, an outbreak in France in 1884 provided samples, which were then to be used for cholera research at Berlin. This European episode provides an interesting glimpse of the famous Pasteur-Koch controversy, that had its origins in historical French-German animosities. Writing to his colleagues in the field investigating the French cholera outbreak Pasteur had this to say [2]:

"I have been thinking a lot about the big differences between your work and Koch's. He has made very strong but premature conclusions, whereas you have been careful and very reserved. What is the story about Koch's comma bacillus?... I haven't seen this organism. Try to find out the fallacy in his story. How do his microscopic preparations differ from yours? He must have made some sort of great error, if he thinks that in cultures from cholera feces he always sees a bacillus which is never seen in ordinary diarrhea. As much as possible, work by yourself. Keep your cadavers to yourself. The reports which you have received that tell you how great this Koch is are wrong. His knowledge of cholera is not that good. If your results agreed with his, he alone would get all the credit. Already, the German newspapers are crowing!"

"... excellent laboratory facilities were placed at their disposal at the Medical College Hospital. The laboratory room that they were given was on the second floor of a building adjacent to the hospital. The room had three large windows so that it was quite favorable for microscopical studies, but the windows were equipped with blinds which could be closed to keep the sun out, so that the room kept fairly cool. A number of tables, chairs, and cabinets were available, making it possible for the group to set up all of their laboratory equipment. The laboratory even had running water and combustion gas. For studies on experimental animals, a stall in the basement was available, and a chemical laboratory was also placed at thier disposal. The autospy room was nearby, so that fresh material could be quickly brought to the laboratory. The only slight disadvantage was that the Medical College Hospital did not have a large number of cholera patients; however, other hospitals in the area provided all that could be desired in that way."[3]

The French newspapers were not far behind as seen from this extract from *La Nouvelle Presse*[2]:

"5 July 1883. *L'affair du Dr. Koch*

We have taken the following from a dispatch from Berlin regarding Dr. Koch's mission to Toulon. "Dr. Koch is going to Toulon at the request of the French government. Because the French Cholera Commission was unsuccessful, the French government has decided to employ exactly Dr. Koch's methods."... It is inconceivable to us that the French government would call into such a mission a Prussian scientist, even one of such scientific authority. France, which has the honor of having in its ranks such eminent savants as M. Pasteur, and which has a Faculty of Medicine renowned throughout Europe... does not have any need for the services of a German scientist. M. Pasteur has made a vast number of discoveries of

> the world of the infinitely small in the past 20 years and is quite able to handle cholera himself."

Koch returned to cholera in 1892 when there was an outbreak at Hamburg and it was here that Koch drew a direct correlation between the purity of water and the incidence of cholera. He unequivocally demonstrated that water filtration was the key to controlling the disease. In his words[2]:

> "For a bacteriologist, nothing is easier than to explain why cholera is restricted to Hamburg. The cholera bacteria are brought into the Hamburg water either from the Hamburg sewers, or from the dejecta of persons living on the boats anchored near where the water is taken .... Altona takes its water from a source which is much worse than Hamburg's, but careful filtration renders it completely, or nearly completely, free of cholera bacteria ...."

Koch was undoubtedly the pioneer in advancing public health measures for the control of disease and in advancing the idea of treating populations instead of individuals. Was Koch treading completely virgin ground in his attack on cholera? W. E. van Heyningen, the historian of cholera, (see page 650 of this issue) suggests that "by the time Koch set off to Egypt to study cholera, everything he was going to learn there was already known and published". The English physician John Snow had already discovered the particulate nature of the cholera poison, while the Italian Filippo Pacini had described the "curved organisms swarming in the intestinal contents of cholera patients, and ascribed the cause of the disease to them, giving them the name *Vibrio cholerae*" in the 1850's. Despite this caveat, Koch's contributions to cholera research were remarkable, although his eminence as a bacteriologist together with his misunderstanding of the symptoms were to lead the field to a blind alley. It was three quarters of a century after the Berlin conferences that Calcutta was once again the scene of a great new insight into the nature of cholera. This time it was Sambhu Nath De and the discovery of the cholera enterotoxin.

P. Balaram is in the Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012.

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Origins of Cholera

There is some disagreement about cholera's antiquity and about its homeland. De, one of India's noted cholera investigators, maintains that cholera is – or rather, was – not an exclusively Asiatic disease. He claims that cholera was present in the classical world and Arabia and in Europe in the seventeenth and eighteenth centuries. Howard-Jones disagrees; he points out that ever since the term "cholera" appeared in Hippocratic writings nearly 2,400 years ago it has, until the late nineteenth century, been a nosological mess, standing for all kinds of ill-defined sporadic gastrointestinal derangements of diverse origins, none of them having any relation to true Indian, or Asiatic, cholera. The fact of the matter, as Greenwood has pointed out, is that while clinical *cases* of European "cholera" may have been very similar to *cases* of real Asiatic cholera, "epidemiologists agreeing in little else agree that the particular manifestations of cholera which showed themselves in Europe after Waterloo differed epidemiologically from anything which had been seen before. This difference may be summarized in a phrase as a difference in *dispersiveness*".

Excerpted from *Cholera, The American Scientific Experience, 1947-1980* by W. E. van Heyningen and J. R. Seal.