no longer exists in nature. The recent sequencing of the genome of horsepoxvirus seems to support the concept that the vaccinia virus may indeed have been derived from horsepox, an origin that Jenner himself suspected (15).

As Jenner demonstrated with cowpox (or horsepox) and smallpox, there are instances among viruses where, because of the host specificity of virulence, an animal virus gives aborted and attenuated infection in the human host, sometimes leading to an acceptable level of protection. Examples include influenza viruses, rotaviruses, and parainfluenza viruses. Remarkable is the fact that the Jennerian approach to immunoprophylaxis remains valid in modern vaccine development, as the reader will see in the chapter ''Vaccines against Rotavirus Gastroenteritis.'' Finally, it is worth noting that Louis Pasteur (Fig. 2), himself one of the most influential pioneers of vaccinology, coined the term ''vaccine,'' in honor of Jenner, to refer generically to immunizing agents.

THE FIRST USES OF ATTENUATED BACTERIA AS PARENTERAL AND ORAL IMMUNIZING AGENTS

In the last quarter of the 19th century bacteriology became a burgeoning science. One after another, bacteria came to be revealed as etiological agents of important human diseases such as cholera, typhoid fever, plague, diphtheria, and tuberculosis and of veterinary diseases such as anthrax and tuberculosis. The ability to obtain pure cultures of the causative bacteria paved the way for the development of vaccines.

Pasteur observed that cultures of *Pasteurella septica*, which causes the lethal disease fowl cholera in chickens, lost their virulence when the cultures were allowed to sit for two weeks (16). He found that chickens inoculated with the old cultures did not develop illness and, furthermore, were protected when subsequently inoculated with highly virulent fresh cultures. Pasteur concluded that in old cultures the bacteria undergo certain changes that result in attenuation but not in loss of immunogenicity.

Pasteur applied his theory in an attempt to attenuate *Bacillus anthracis*, the cause of anthrax, an infection of cows, sheep, and goats (and occasionally humans) that is often fatal. Pasteur found that maintaining shallow cultures of *B. anthracis* at a temperature of 42°C to 43°C for two weeks resulted in a loss of virulence (furthermore, spores did not form at this temperature). In these early experiments, Pasteur established an approach that was followed in multiple later attempts at immunoprophylaxis: the first inoculations given in the immunization schedule were highly attenuated to maximize safety, whereas subsequent inoculations were somewhat less attenuated to increase antigenicity.

On May 5, 1881, Pasteur and his colleagues Emile Roux and Charles Chamberland carried out a historic public experiment in Pouilly-le-Fort, France (17,18). They inoculated one set of farm animals (24 sheep, 1 goat, and 6 cows) with an initial highly attenuated vaccine; a second inoculation with a less-attenuated vaccine preparation was given on May 17. On May 31, these immunized animals and a set of uninoculated controls (24 sheep, 1 goat, and 4 cows) were challenged with virulent *B. anthracis*. Over the next four days, spectacular results documented the efficacy of the vaccine: in the control group, the 24 sheep and the goat died and the four cows became overtly ill, whereas there was only one death (a sheep) among the vaccinated animals. Within a short time, the anthrax vaccine became widely used in France. As early as 1882, Pasteur was able to report excellent results from the use of the vaccine in more than 79,000 sheep in France (19).

The first bacterial vaccine used in humans was administered in 1884, barely one year after the initial isolation of *Vibrio cholerae* by Robert Koch (20), when Jaime Ferran inoculated live, putatively weakened, *V. cholerae* parenterally (21). Ferran's vaccine, which consisted of broth cultures containing ''attenuated'' vibrios, was given to about 30,000 individuals who eagerly sought protection during the 1884 epidemic of cholera in Spain. This experience generated much interest internationally, and commissions from several countries came to inspect and evaluate Ferran's work. The most influential committee, sponsored by the Pasteur Institute, Paris, criticized Ferran's vaccine and argued that no convincing proof was provided to support claims for a prophylactic effect (22). Furthermore, it was reported that Ferran's live vaccine was heavily contaminated with other microorganisms and that only a small proportion of the bacteria were *V. cholerae* (22); contamination may have accounted for the severe adverse reactions associated with this vaccine and its apparent lack of efficacy (22).

In 1891, only one year after Waldemar Haffkine had joined the Pasteur Institute in Paris, Pasteur asked him to carry out research to develop an immunizing agent against cholera (23). Following Pasteur's general principle that live vaccines confer protection superior to vaccines consisting of killed microorganisms, Haffkine prepared two modified *V. cholerae* strains for use as live vaccines. The first strain was attenuated by culture at 39°C with continuous aeration, whereas the second strain underwent multiple intraperitoneal passages in guinea pigs in an attempt to increase its virulence. Haffkine utilized these strains sequentially as parenteral immunizing agents; the attenuated strain was inoculated first, followed six days later by the strain of supposedly enhanced virulence (24). Typical side reactions following vaccination included fever, malaise, and headache as well as pain and swelling at the injection site. In later evaluations of the vaccine, Haffkine abandoned the initial inoculation with the attenuated vibrio and administered to humans only the pathogenic strain without an increase in adverse reactions. Statistical analysis of several clinical trials of Haffkine's live cholera vaccine in India suggested that it was efficacious (25). Nevertheless, further use of the vaccine was abandoned because of difficulty in standardizing it and producing it in large quantities.

The other early success in attenuated bacterial vaccines was the bacille Calmette-Guerin (BCG) vaccine against tuberculosis. Leon Charles Albert Calmette and Jean-Marie Camille Guerin obtained a stable, attenuated strain incapable of causing tuberculosis in the highly susceptible guinea pig (26). It was achieved by repeatedly subculturing (213 times over 13 years), in the presence of ox bile, a tubercle bacillus originally isolated from a cow by Edmond Nocard in 1902 (26). The first administration of BCG vaccine to a human occurred in 1921, when a newborn infant, whose mother had died of tuberculosis, was given an oral dose without adverse effects. Calmette initially advised that the vaccine should be administered orally to young infants. Accordingly, by the late 1920s, approximately 50,000 French infants had received the apparently well-tolerated BCG vaccine (27). By the late 1920s, the intradermal route of inoculation rapidly began to replace the oral route of vaccination. It was not until the 1950s that controlled field trials confirmed the efficacy of at least one strain of BCG (28).