Figure 4 Diphtheria toxoid production at the CSL, Parkville, Victoria, Australia, 1929. *Abbreviation*: CSL, Commonwealth Serum Laboratories. *Source*: Photo courtesy of CSL.

use of toxin/antitoxin mixtures to achieve active immunization (55). As will be reviewed later, mistakes in the preparation of such mixtures sometimes led to disastrous consequences. Ultimately, it was found that by treatment with formalin, diphtheria and tetanus toxins could be rendered biologically innocuous yet retain their ability to stimulate neutralizing antitoxin (56,57). Alexander Glenny (57) claims to have prepared formalinized diphtheria toxoid as early as 1904. In a personal communication to Henry Parish (1), Glenny related that his first toxoid was prepared by accident! He observed that a batch of diphtheria toxin lacked toxic activity yet elicited antitoxin in animals as efficiently as fully active toxin. The *C. diphtheriae* cultures used to produce the toxin had been grown in large earthenware containers that could not be readily sterilized. One of the steps in sterilizing the containers for the next batch involved washing them with formalin. Glenny hypothesized that residual formalin had apparently inactivated the toxin. He subsequently proved that formalin could, indeed, alter diphtheria toxin to toxoid, rendering it innocuous yet preserving its antigenicity (Fig. 4).

STIMULATION OF LOCAL IMMUNITY

Many infectious agents interact with the mucosa of the gastrointestinal tract, the respiratory tract, or the urinary tract as a site of colonization or as a preliminary step before invasion. Recognition of the mucosal immune system as a unique component of the overall immune system of the mammalian host underlies extensive current research to develop oral or intranasal vaccines to prevent enteric infections such as cholera, typhoid fever, rotavirus diarrhea, and shigellosis and respiratory infections such as influenza and RSV bronchiolitis. The leading pioneer in the concept of local immunization was Alexandre Besredka (58), who was generations ahead of his time in his approach and his concepts. Besredka, however, did not believe that antibodies were involved in mediating the local immunity that he stimulated. Albert B. Sabin's pioneering work resulting

Figure 5 Albert B. Sabin (1906–1993), a 20th century pioneer of vaccinology, administering oral polio vaccine in 1959. *Source*: Photo courtesy of Heloisa Sabin.

in a practical and effective live oral vaccine against poliomyelitis set a paradigm for other oral and nasal vaccines (Fig. 5).

The parts of this book describing vaccines against cholera show that the modem approach to prevention of this disease involves oral immunization with either inactivated antigens or attenuated bacteria. However, these modern oral cholera vaccines are descendants of a long tradition. For example, the first report of nonliving whole *V. cholerae* used as an oral vaccine in humans was published in 1893 (59); this report related the lack of adverse reactions following ingestion of multiple doses containing billions of inactivated vibrios.

In the 1920s and 1930s, field trials of Besredka's killed oral *V. cholerae* vaccine, combined with bile (so called ''bilivaccine''), were carried out in India (60–62) and Indochina (63). Significant protection was apparently achieved. In the Indian trials, the oral vaccine was also compared with a killed parenteral whole-cell vaccine. However, it is not certain that the vaccine and control (nonvaccinated) groups were fairly randomized so that the risk of infection was equal. Nevertheless, the oral bilivaccine provided 82% protection and the parenteral vaccine 80% protection during the period of surveillance (60–62). The bilivaccine was administered in a total of three doses on consecutive days; the subject to be vaccinated first ingested a bile tablet, followed, 15 minutes thereafter, by a bilivaccine tablet containing 70 billion dried vibrios. Because of the bile component, the bilivaccine commonly caused adverse reactions, including nausea, vomiting, and acute diarrhea; it appears