SPECIAL ARTICLE

David Bodian’s Contribution to the Development of Poliovirus Vaccine

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Abbreviation: MV, mixed virus.

David Bodian spent almost his whole scientific career (1942–1983) at the Johns Hopkins University, first in the Department of Epidemiology at the School of Hygiene and Public Health and then in the Department of Anatomy at the School of Medicine. Arguably, his most important research contribution was the elucidation of the pathogenesis of poliomyelitis—a contribution that played a major role in the development of inactivated poliovirus vaccine (Salk vaccine), the first successful vaccine against poliomyelitis. In this historical review, I have chosen to focus on those aspects of Bodian’s research that were relevant to vaccine development. This retelling of the story is necessarily biased, since it emphasizes the work of one investigator while acknowledging that less attention is paid to the work of many others who made important contributions. In addition, I distort history somewhat by presenting the research results in an apparently logical sequence, while in truth the story more resembled a jigsaw puzzle put together somewhat randomly to reveal an orderly whole when completed. Furthermore, I have deliberately chosen some examples from my own collaborations with David Bodian, even though the experiments were performed after 1955. Finally, as Dave Bodian’s last trainee in virology, I write this with fond memories of an inspiring role model.

I begin with a quotation from a talk that David Bodian gave in 1976, at the time of his retirement as chair of the Department of Anatomy (1): “In 1945, Professor Burnet of Melbourne wrote, ‘While I was in America recently I had good opportunity to meet with most of the men actively engaged on research in poliomyelitis... The part played by acquired immunity to poliomyelitis is still completely uncertain, and the practical problem of preventing infantile paralysis has not been solved. It is even doubtful whether it ever will be solved.’ ... Most of us doing research on poliomyelitis in 1945 were mainly motivated by curiosity, rather than by the hope of a practical solution in our lifetime.” And yet, on April 12, 1955, just 10 years after Burnet’s 1945 letter, Thomas Francis announced the successful field trial of inactivated poliovirus vaccine (2). What explains this abrupt transition from a state of confusion and dismay to the triumphant optimism of 1955? The explanation lies in a set of discoveries that were made regarding the pathogenesis of poliovirus infection and the role of antibody in its control. This story is the subject of the following essay.
TABLE 1. The scientific path to the development of poliovirus vaccine, 1949–1955

<table>
<thead>
<tr>
<th>Advance</th>
<th>Date</th>
<th>Investigator(s) (partial listing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture of poliovirus</td>
<td>1949</td>
<td>Enders</td>
</tr>
<tr>
<td>Creation of monkey model using field isolates of poliovirus</td>
<td>1950s</td>
<td>Bodian, Horstmann</td>
</tr>
<tr>
<td>Viremia in nonhuman primates</td>
<td>1950s</td>
<td>Bodian, Horstmann</td>
</tr>
<tr>
<td>Viremia in humans</td>
<td>1954</td>
<td>Bodian, Horstmann</td>
</tr>
<tr>
<td>Integrated pathogenesis summary</td>
<td>1955</td>
<td>Bodian, Sabin</td>
</tr>
<tr>
<td>Assay for neutralizing antibody in cell culture</td>
<td>1950s</td>
<td>Salk</td>
</tr>
<tr>
<td>Identification of the three serotypes of poliovirus</td>
<td>1950s</td>
<td>Bodian, Salk</td>
</tr>
<tr>
<td>Termination of viremia by antibodies</td>
<td>1950s</td>
<td>Bodian, others</td>
</tr>
<tr>
<td>Prevention of paralysis by passive antibodies</td>
<td>1950s</td>
<td>Bodian, Howe</td>
</tr>
<tr>
<td>Neutralizing antibodies induced by inactivated poliovirus</td>
<td>1950s</td>
<td>Salk</td>
</tr>
<tr>
<td>Vaccine protection shown to be correlated with antibody level in prospective human trial</td>
<td>1955</td>
<td>Francis</td>
</tr>
</tbody>
</table>

PATHOGENESIS OF POLIOMYELITIS

The key discoveries leading to the formulation and testing of the first poliovirus vaccine are summarized in table 1. The first breakthrough was the finding that primary cultures of human cells could be used to grow poliovirus and that the virus produced a rapid, consistent, and readily detected cytopathic effect (3). This finding provided a system for the ready isolation of poliovirus from patients—a method of growing virus stocks, quantitating infectious virus, and measuring neutralizing antibody. Simple as it may have been, the cell culture system had profound implications for research on poliomyelitis. First, it led to the isolation of “wild-type” strains of poliovirus directly from the stools and throat swabs of infected patients; these low-passage isolates were used to prepare virus stocks that could be used for experimental studies. Prior to this time, experimental virus stocks had been prepared from the spinal cords of monkeys infected by intracerebral injection of the virus, and the “standard” virus used for most studies was the mixed virus (MV) strain. The MV strain had been developed at the Rockefeller Institute in the laboratory of Simon Flexner and had undergone many serial intracerebral passages.

When virus isolates obtained by Enders’ cell culture method were compared with the MV strain, they were found to differ dramatically in their biologic properties. Multiple intracerebral passages of the MV strain had selected for a virus that was highly neurotropic and did not cause viremia, while fresh wild-type isolates were viremogenic.

Table 2 illustrates these differences and contrasts neuroadapted (MV strain) and viremogenic (Mahoney strain) poliovirus (4). In this experiment, cynomolgus monkeys were divided into two groups; one group underwent a nerve block by freezing of the sciatic nerve and the other group had a sham operation. Both groups were then given poliovirus injections in the gastrocnemius (calf) muscle. Nerve block protected against the neurotropic MV strain but not against the Mahoney strain, indicating that the Mahoney virus spread via the bloodstream—bypassing the neural block—while the MV strain could not bypass the neural block, since it was an obligatory neurotrope.

Concepts of the pathogenesis of poliomyelitis had been based on monkey experiments with the MV strain, and those concepts had to be radically revised on the basis of experiments with wild-type isolates. Central to the revised view of poliovirus pathogenesis was the role of viremia.

Figure 1 shows an experiment in which cynomolgus monkeys were inoculated intravascularly with four wild-type isolates (5). Each of the isolates induced viremia, but there was marked difference in the viremogenic potential of different isolates. Three caused minimal viremia that was detectable only through testing at multiple time points after infection and led to a low frequency of paralysis. By contrast, the Mahoney strain—which produced the highest-titer viremia— Paralyzed about 50 percent of infected monkeys. The correlation of viremia level and paralysis rate provided important circumstantial evidence for the role of viremia in the path to the central nervous system (5).

Parallel investigations conducted during outbreaks of paralytic poliomyelitis showed that viremia also occurred in human infection. Table 3 shows results from a study in which contacts of patients with paralytic poliomyelitis were bled twice, shortly after the onset of the index case and about

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Neuroadapted MV strain</th>
<th>Viremogenic Mahoney strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25/26</td>
<td>19/19</td>
</tr>
<tr>
<td>Nerve block</td>
<td>24/0/11</td>
<td>3/5</td>
</tr>
<tr>
<td>Injected leg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1/3</td>
<td>16/13</td>
</tr>
<tr>
<td>No. of days from incubation to paralysis</td>
<td>5/5</td>
<td>7/7.5</td>
</tr>
</tbody>
</table>

* MV, mixed virus.
† See text for explanation. Data were obtained from the paper by Nathanson and Bodian (4).
‡ Not applicable.

Am J Epidemiol 2005;161:207–212
1 month later (6). The paired serum samples were tested for antibody, and the first serum samples taken were also tested for virus. Contacts were classified in several categories: those previously infected with poliovirus; those who were not infected; those with recent infection (converters) who had antibody in the first serum sample; and those converters with no detectable antibody in the first serum sample. There were nine subjects in the last category, and virus was isolated from five of those nine, which is a significant success rate considering the single sample tested and the low level of viremia produced by many wild-type poliovirus strains (figure 1).

The reconstructed view (figure 2) of pathogenesis indicated that poliovirus was an enterovirus that initially infected lymphoid tissues in the tonsil and intestine (Peyer’s patches) and then spread to draining lymph nodes, and—via lymphatic drainage—entered the circulation (7). Bloodborne virus invaded the central nervous system, either directly across the blood-brain barrier or via peripheral nerve ganglia, and resulted in destruction of lower motor neurons in the spinal cord, producing the flaccid paralysis characteristic of poliomyelitis. The critical point was that viremia was an essential step on the pathway from portal of entry to the central nervous system.

### PROTECTIVE ROLE OF ANTIBODY AND FORMULATION OF A VACCINE

The revised view of the pathogenesis of poliomyelitis set the stage for studies on the role of antibodies in recovery from poliovirus infection and protection against paralytic poliomyelitis. A necessary preliminary to these studies was a simple, quick, and reproducible test for neutralizing antibodies. This was the metabolic inhibition test developed by Jonas Salk, based on Enders’ cell culture system. As practiced in the Bodian laboratory in the 1950s (8), the metabolic inhibition test utilized small test tubes in which serial dilutions of serum samples were set up in nutrient medium plus phenol red indicator dye. A virus inoculum of 100 tissue culture infectious doses was added; the mixture was left at room temperature for 1 hour to permit neutralization to occur; and a freshly prepared suspension of monkey kidney cells was added. After 5 days of incubation at 37°C, the test was read. In the absence of virus, the cells produced metabolic acid and the phenol red dye turned yellow; if virus was present, the cells were destroyed and the color remained red; and if antibody neutralized the virus, the cells metabolized and the culture was yellow. Using this system, it was possible to obtain very clear endpoints within a few twofold dilutions of the test serum.

The other critical prerequisite for the rational development of a vaccine was determination of whether there was a single antigenic type of the virus (as with measles and smallpox viruses) or several antigenic types (as with adenoviruses and rhinoviruses). For this purpose, antigenic similarity or difference was best defined as cross-protection in humans. In other words, if a single infection with wild-type poliovirus consistently protected an individual against the paralytic consequences of subsequent exposure to poliovirus, it could be considered that all strains of the virus were part of a single antigenic type. On the other hand, if there were several different antigenic types, a putative efficacious vaccine formulation would need to include each of these types.

There were reported anecdotal instances of patients who had experienced more than a single paralytic attack from poliomyelitis, but they were rare and had not been well documented in the absence of the ability to isolate virus and test patients for neutralizing antibody. Therefore, the most rigorous approach to this question was to infect monkeys with a given strain of poliovirus and, a few months later, challenge them with a second strain.

Table 4 provides an extract from such an experiment, which differentiated two antigenic types of poliovirus (9). In this experiment, rhesus monkeys were first infected with one of four viral isolates. Many of the animals developed paralysis but survived, and the surviving paralyzed monkeys were then challenged by a second intracerebral injection with either the same isolate or a different isolate. Homologous challenge (reinfection with the same virus) provided 100 percent protection, indicating absolute protection with viruses in the same antigenic group. Using this standard, if

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**Table 3. Occurrence of viremia in human poliovirus infection**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paralytic index cases</td>
<td>47</td>
</tr>
<tr>
<td>Household contacts aged 1–13 years</td>
<td></td>
</tr>
<tr>
<td>Paired sera: first 2–12 days after onset of index case</td>
<td>84</td>
</tr>
<tr>
<td>Prior infection: first serum antibody titer &gt;100</td>
<td>50</td>
</tr>
<tr>
<td>No infection: second serum antibody titer &lt;2</td>
<td>6</td>
</tr>
<tr>
<td>Recent infection: first serum antibody titer 2 or 4</td>
<td>19</td>
</tr>
<tr>
<td>Recent infection: first serum antibody titer &lt;1</td>
<td>9</td>
</tr>
<tr>
<td>Virus isolated</td>
<td>5/9 (56%)</td>
</tr>
</tbody>
</table>

* See text for details. Data were obtained from the paper by Bodian and Paffenbarger (6).
the first infection conferred 100 percent protection against a different virus isolate (heterologous challenge), the two viruses were classified in the same antigenic group. If the second virus caused a second paralytic attack in some of the animals (partial cross-protection), it was classified in a different antigenic group. On these grounds, the four strains compared in table 4 could be placed into two antigenic groups.

Experiments of this kind, done by Bodian and others, indicated clearly that wild-type polioviruses could be classified into three types, as defined by cross-protection. Parallel results were obtained using Salk’s neutralization test. These data implied that a protective vaccine would have to be multivalent—that is, it would have to immunize against each of three antigenic types.

The similarity of results using cross-challenge of monkeys and tests of serum neutralizing antibody led to the question

![Figure 2](https://academic.oup.com/aje/article-abstract/161/3/207/126890)

**FIGURE 2.** David Bodian’s scheme of the pathogenesis of poliovirus infection based on studies in monkeys, chimpanzees, and humans. Reproduced with permission from the original article by Bodian (7). (Copyright 1955, American Association for the Advancement of Science (http://www.sciencemag.org).) CNS, central nervous system.

![Figure 3](https://academic.oup.com/aje/article-abstract/161/3/207/126890)

**FIGURE 3.** Apparent termination of viremia by neutralizing antibodies (see discussion in text). Data were obtained from the paper by Nathanson and Bodian (8). TCD50, 50% tissue culture infectious dose.

### TABLE 4. Differentiation of two antigenic types of poliovirus by intracerebral challenge

<table>
<thead>
<tr>
<th>First paralytic infection (1,000 intracerebral PD50)</th>
<th>Second infection (10,000 intracerebral PD50) 5-23 weeks after first infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lansing</td>
</tr>
<tr>
<td>Type 2</td>
<td></td>
</tr>
<tr>
<td>Lansing</td>
<td>0/6</td>
</tr>
<tr>
<td>Type 1</td>
<td></td>
</tr>
<tr>
<td>Brunhilde</td>
<td>8/20</td>
</tr>
<tr>
<td>Kotter</td>
<td>4/11</td>
</tr>
<tr>
<td>Frederick</td>
<td>19/33</td>
</tr>
<tr>
<td>Normal controls</td>
<td>26/28</td>
</tr>
</tbody>
</table>

* See text for explanation. Data were obtained from the paper by Bodian (9).
† PD50, 50 percent paralytic dose.
of the role of antibody in recovery from poliovirus infection. Figure 3 models acute poliovirus infection of humans, using a virulent wild-type virus in monkeys (8). In this experiment, cynomolgus macaques were infected intramuscularly with the Mahoney strain of poliovirus and were followed for viremia and the appearance of neutralizing antibodies in their serum. Mahoney virus induced viremia that had its onset a few days after infection and lasted less than 1 week. Importantly, immediately following the disappearance of virus from the blood, neutralizing antibody was first detected and rose in titer thereafter. The detection of antibody on the first day after viremia disappeared was consistent with the interpretation that it was responsible for ending the viremia phase.

Evidence that antibodies played a critical role in the termination of viremia suggested that circulating antibodies might be able to prevent viremia if they were present prior to infection. Figure 4 shows results from an experiment in which this hypothesis was tested (10). Cynomolgus monkeys were given graded doses of antibody (pooled human gamma-globulin) by intramuscular injection; 24 hours later, the animals were bled and the serum was tested for the level of passive antibody. Right after bleeding, the monkeys were given an injection at a different intramuscular site with a paralyzing dose of Mahoney virus. Assays of serum obtained prior to viral infection showed that each twofold increase in the dose of antibody produced a corresponding twofold increase in the level of antibody in the recipient monkeys. Furthermore, there was a strong correlation between passive antibody level and degree of protection against paralytic poliomyelitis. Thus, in the control group given no antibody, the rate of paralysis was approximately 95 percent; an antibody titer of 3 conferred little protection; a titer of 6 conferred about 50 percent protection; and a titer of 11 conferred about 90 percent protection. This result implied that neutralizing antibody could provide protection against potentially paralytic infection with poliovirus.

Data of this type made available a "correlate of protection," one that could be quantified. By inference, if an immu-

![Figure 4](https://example.com/figure4.png)

**FIGURE 4.** Protection against potentially paralytic poliovirus infection provided by neutralizing antibodies (see text for description of experiment). Data were obtained from the paper by Nathanson and Bodian (10).

![Figure 5](https://example.com/figure5.png)

**FIGURE 5.** Neutralizing antibody response of normal human volunteers to immunization with an experimental batch of inactivated poliovirus vaccine (see text for details). Reproduced with permission from the original article by Salk (11). TCID<sub>50</sub>, 50% tissue culture infectious dose.

**TABLE 5.** Correlation between titer of neutralizing antibody and protection against paralytic poliomyelitis in recipients of inactivated poliovirus vaccine

<table>
<thead>
<tr>
<th>Immunization status</th>
<th>Parameter</th>
<th>No. or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unvaccinated control</td>
<td>No. of paralytic cases observed</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Percentage &lt;1:8</td>
<td>35.4</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>No. of paralytic cases expected at &lt;1:8</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>No. of paralytic cases observed</td>
<td>14</td>
</tr>
</tbody>
</table>

* See text for explanation. Data were obtained from the paper by Francis et al. (12).
nogen could be prepared that would induce neutralizing antibodies in the circulation, it might protect immunized subjects against the paralytic consequences of subsequent exposure to wild-type poliovirus. It was now timely to consider various ways in which poliovirus or its antigens could be formulated as a vaccine, using the correlate of protection to evaluate potential efficacy. Jonas Salk (and later Albert Sabin) relied heavily upon this correlate as he evaluated different experimental vaccine formulations. Salk found that a simple chemical, formaldehyde, could be used to inactivate poliovirus and render it noninfectious in both cell cultures and animals. Furthermore, when injected intramuscularly, this inactivated poliovirus vaccine would elicit neutralizing antibodies.

Figure 5 shows results from a trial of the immunogenicity of an experimental batch of inactivated poliovirus vaccine (11). The subjects had preexisting antibody to none, one, or two types of poliovirus prior to immunization with a trivalent vaccine. Responses shown are responses only to the serotypes that were negative prior to immunization. The experimental vaccine induced an immune response in all individuals and to all three serotypes of the virus; furthermore, antibody responses in most participants exceeded the critical level of 10 by a considerable margin. Data of this kind provided significant promise of the efficacy of such a vaccine. (This brief account bypasses all of the complex issues surrounding safety and production of inactivated poliovirus vaccine.)

To test the protective efficacy of inactivated poliovirus vaccine, a large field trial was conducted in 1954 (12). This trial involved hundreds of thousands of children (selected as the highest-risk group) and included a vaccinated group and a placebo control group. No attempt is made here to describe this massive trial, which stands as a landmark in human experimentation. I will only refer to one data set extracted from the published results (12). From the trial data, it was possible to test the hypothesis that the putative correlate of protection (a neutralizing antibody titer of about 10) would protect against paralytic poliomyelitis.

Table 5 shows that, in the placebo control group, there were 40 cases of paralytic poliomyelitis (12). The hypothesis was formulated that vaccinees with a neutralizing antibody titer of 8 or greater would be protected and those with a titer of less than 8 would not. Among vaccinees, 35.4 percent had titers less than 8, leading to the prediction of 14.2 paralytic cases among vaccinees (35.4 percent of 40 placebo cases). In fact, 14 cases were observed in the vaccinated group, confirming the hypothesis and validating neutralizing antibody as a correlate of protection.

REPRISE

This account is admittedly both brief and oversimplified. However, I believe it documents the critical contribution of research on the pathogenesis of poliomyelitis to the development of an efficacious poliovirus vaccine. I have tried to indicate the central role that David Bodian played in elucidating the pathogenesis of poliomyelitis and how his discoveries moved the field forward in a logical progression toward the formulation and testing of inactivated poliovirus vaccine. Once again, I emphasize that David Bodian was part of a larger group of investigators whose aggregate contributions were essential to the success of the undertaking. However, I would venture, as an admittedly biased observer, to assert that Dave was clearly one of the intellectual leaders of this effort, a scientist to whom others turned because of his keen mind, analytic prowess, and recognized integrity.

REFERENCES