RESPONSE OF CHICKS TO POLIOVIRUS ANTIGENS

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THE chicken is capable of producing antibodies to the three antigenic types of poliovirus (*1–4*). Only limited virus multiplication occurs in this host's tissue (*5,6*), but the response to both live and inactivated virus appears to be adequate for quantitative estimation of antigenic potency (*3,7*). Advantages of using young chickens instead of the commonly used mammalian species include less cost per chick, relatively large numbers held in a relatively small space, simplified maintenance, and ready availability from commercial sources. The use of the chick for determining the antigenicity of poliovirus antigens has been discussed recently by Mascoli (*3*).

Since the chick has been suggested by Timm, Rope, and McLean (*4*) as a reliable animal for determining the capacity of a poliovirus antigen to produce specific antibodies, we decided to examine further certain aspects of this response. The primary objectives of the studies reported here were: (*a*) to examine the time interval required to obtain an antibody response following injection of poliovirus antigens, (*b*) to determine the optimal interval between the first and second injection, (*c*) to examine the effect of age difference on the capacity to respond to the same dose of antigen, and (*d*) to determine the response of chicks to poliovirus antigen combined with diptheria, tetanus, and pertussis (DTP) antigens.

Materials and Methods

Chicks. Most of the birds used in these experiments were of the "Hy-line" strain. They were obtained from a commercial hatchery at 1 to 3 days of age and kept in electric brooders for 6 to 8 days before being used in experiments. A few laying hens also were used. All birds were fed a commercial feed according to the manufacturer's recommendations.

Antigens. Polyvalent, inactivated antigens in the form of poliovirus vaccines were prepared by the usual methods used for vaccine production. They contained the following viruses: type 1 Mahoney, type 2 MEF_1, and type 3 Saukett. Formalin inactivation was used in all experiments. No adjuvant was added.

Immunization. The poliovirus antigen preparations were administered by intramuscular injection in all experiments. For chicks less than 2 weeks of age, the dose was divided so that 0.5 ml. was given in each leg. Older birds were given the full dose of 1.0 ml. in one leg. Usually two injections were given, one at the start of the experiment and the second 14 days later.

To examine the effects of varying the intervals between injections, intervals of 4, 6, 8, 10, 12, and 14 days were tried. Blood samples from the animals were obtained by cardiac puncture either 7 days after the last injection or, in a few cases, at varying intervals after the primary or secondary injection. The serum was separated and stored at —17° C. until tested.

Control monkeys were given three injections of 1 ml. each, 7 days apart, and blood samples were taken 7 days after the last injection.

Antiserum titrations. A modification of the pH metabolic inhibition test, described by Salk and associates (*7*), was used to determine serum antibody concentrations. Several antiserum

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titration methods were employed: (*a*) serial twofold dilutions of antiserums were prepared and the geometric mean titers of viral inhibition were calculated, and (*b*) serums were diluted 1:8 only and the ratio of the number of serums inhibiting viral activity to the number of serums tested was calculated. Serum dilutions were tested against approximately 100 TCD_{50} of appropriate poliovirus type and inhibition of viral activity was determined in the metabolic test.

Antigen titrations were performed by a dose-response method in which serial tenfold dilutions of antigen were injected into groups of 20 chicks and the antiserum of each chick tested for viral inhibition at 1:8 dilution. The reciprocal $\log_{10}$ of the antigen dilution which evoked antibody in 50 percent of the chicks could then be calculated (ED_{50}).

Controls were run with all tests to determine (*a*) serum toxicity to cells, (*b*) actual titer of virus used in the test, (*c*) titers of known positive control serums, and (*d*) normal cell activity. Chick control serum used in the test was obtained by pooling individual serums of birds injected with a standard reference antigen used in this laboratory.

Quadruple vaccine. Poliovirus antigen combined with DTP antigens used in this study have been described previously (*8*). The dosage was adjusted to provide the same amount of poliovirus antigen as in the poliovirus vaccine samples studied. The same lot of poliovirus was used in the preparation of vaccines for comparative testing.

Figure 1. Distribution of reactions of chicks to type 1 poliovirus antigen in seven lots of experimental vaccine

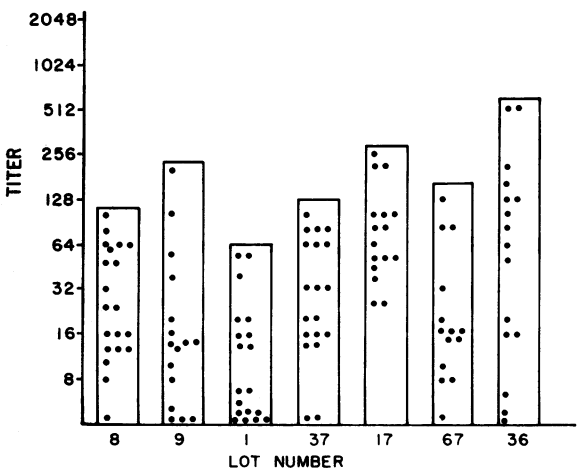


Figure 2. Distribution of reactions of chicks to type 2 poliovirus antigen in seven lots of experimental vaccine

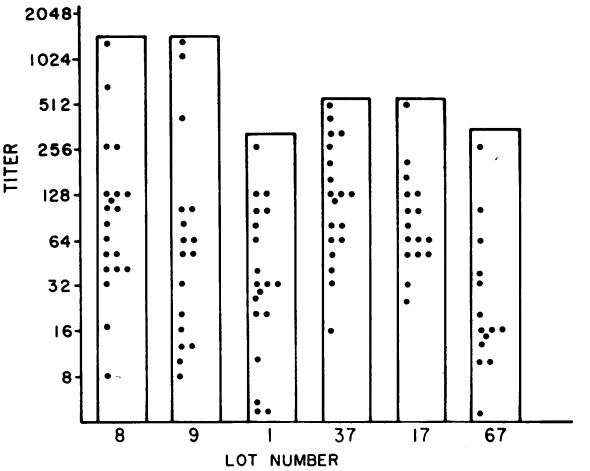
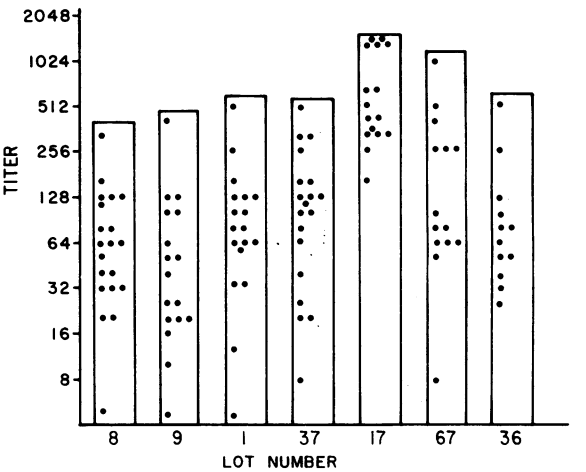


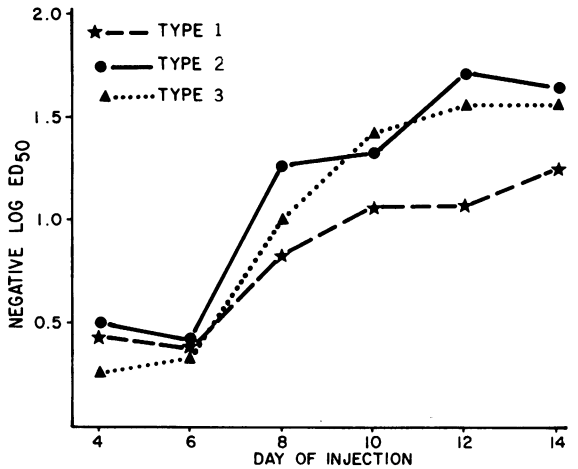
Figure 3. Distribution of reactions of chicks to type 3 poliovirus antigen in seven lots of experimental vaccine



Results

The geometric mean titers of seven poliovirus vaccine samples were determined by chick assay method in seven lots of chicks. Each lot consisted of 15 to 20 chicks. Four of these vaccines were compared in both monkey and chick tests. Only undiluted vaccine was used for monkey injections. Results of comparison

Figure 4. Reactions of chicks to second dose of trivalent poliovirus vaccine injected at various intervals of time [1]



[1] First injection on 0 day; all chicks received 1 ml. each injection; blood samples were taken fifth day after second injection.

NOTE: ED_{50}=antigen dilution producing neutralization of approximately 100 ID_{50} poliovirus by 50 percent of chick serums (1:8 dilution) tested.

tests with monkeys and chicks were similar to those obtained previously (*4* and unpublished data of J. E. Prier, 1958, which are included in data on chick potency testing accumulated by the Division of Biologics Standards, National Institutes of Health).

Distribution of some of the serum titers of chicks injected with undiluted antigen in the various tests are shown in figures 1–3. Results of these experiments approximated those of Timm and associates (*4*) and essentially confirmed their data.

Development of titers. Blood samples were drawn from groups containing five chicks each at intervals following injection of trivalent poliovirus vaccine. Responses were rapid, reaching a peak for all three types of poliovirus vaccine by 72 hours, but antibody levels declined rapidly. At 192 hours after injection, no detectable antibody against types 1 and 3 was observed.

To examine the effect of a booster dose of vaccine at a point when antibody levels are declining, a second dose of vaccine was given to another group of chicks 96 hours after the primary injection. A response similar to that obtained after the primary injection was observed.

Another series of experiments was run to determine the antibody levels at varying intervals following two injections of vaccine, 14 days apart. Although the peak response occurred at about the same time as that following a single injection, the decline in antibody levels was much slower, based on conversion rates.

Effect of varying time between antigen doses. The second dose of vaccine (undiluted) was given at varying intervals after the primary injection. Maximum titers (fig. 4) were obtained in chicks when the full 14 days were allowed between the first and second injections.

Age of chicks. In a comparison of 1- and 4-week-old chicks there was no significant difference in response to the injection of poliovirus vaccine between the two groups. The recent work of Furesz and Moreau (*9*) demonstrates that 6- to 12-month-old chickens respond adequately to single injections of the three types of poliovirus.

Response following injection with poliovirus-DTP antigens. The results of poliovirus and quadruple vaccine injections in chicks, using a dose-response method, are shown in table 1.

Table 1. Effect of bleeding time on antibody response of chicks to second injections of poliovirus and DTP-poliovirus vaccines

Bleeding time (days)	DTP-poliovirus vaccine types			Poliovirus vaccine types		
	1	2	3	1	2	3
1	[1] 0	0	0	0	0	0
3	1. 040	1. 527	1. 407	1. 365	1. 635	1. 635
5	1. 180	1. 430	1. 872	1. 620	1. 732	1. 866
7	. 608	. 707	1. 373	1. 072	1. 356	1. 474
9	0	0	. 172	. 550	. 700	. 694

[1] Minus log ED_{50}.

Two injections of vaccine, 0 and 14 days, were used, and blood samples were drawn at 1, 3, 5, 7, and 9 days after the second injection. The results indicated depression of the poliovirus antigen activity in the mixed vaccine. With both vaccines, however, the optimum bleeding time was 5 days after the second injection.

To determine whether the interval between vaccine injections influenced the validity of the test as applied to quadruple vaccine, the second dose was given from 4 to 21 days after the first. A comparison of the two vaccines is shown in table 2. Blood samples were drawn from all animals 5 days after the second injection. It appears that the dose interval resulting in serologic response to poliovirus antigen differs between single and multiple antigen preparations. Addition of the toxoid and pertussis components results in a preparation that requires a somewhat greater dose interval to measure optimal antibody response.

Discussion

The results of injection of chicks with poliovirus antigen confirm the data previously reported by Timm and associates (*4*). Both studies have shown that the optimum bleeding time for determining the maximum antibody response is approximately 5 days after the second injection. Responses following single injection of antigen were also similar, except that the present studies demonstrated a higher response to type 3 virus. This merely may be a reflection of differences in total antigen concentration in the virus fluids used.

When the chick poliovirus potency test is applied to preparations containing tetanus and diphtheria toxoids and pertussis bacterin, the peak antibody level is reached at about 5 days after the second injection. This, therefore, approximates the optimal bleeding time for birds receiving only poliovirus antigen.

The antibody response, as it relates to the interval between the two antigen doses, differs between the two vaccines. With poliovaccine alone, the response is detectable in an interval of 4 days and increases as the time between injections approaches an optimum of 14 days. Quadruple vaccine requires a greater interval and the optimum period extends beyond 14 days. To assure detection of maximum response, therefore, it seems advisable to increase the dose interval to 17 days for testing chicks with quadruple antigen.

Responses to single doses of poliovirus antigen confirm the work of others (*2,4*) and further suggest this procedure as an effective test for evaluation of poliovirus antigens.

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Table 2. Effect of altered dose interval on antibody response of chicks to second injection of poliovirus and DTP-poliovirus vaccines

Dose interval (days)	DTP-poliovirus types			Poliovirus vaccine types		
	1	2	3	1	2	3
4	0	0	0	[1] 0. 45	0. 5	0. 25
6	0	0	0	. 3	. 35	. 3
8	. 03	. 09	. 4	. 85	1. 23	1. 01
10	. 51	. 41	. 83	1. 05	1. 31	1. 36
12	. 75	1. 4	1. 49	1. 05	1. 7	1. 55
14	1. 32	1. 57	1. 72	1. 15	1. 6	1. 55
10	. 09	. 08	. 81	1. 1	. 8	1. 25
14	. 49	1. 63	1. 95	1. 26	1. 46	1. 77
17	1. 44	1. 53	1. 92	. 95	1. 26	1. 3
21	1. 45	1. 6	1. 95	1. 2	1. 87	1. 8

[1] Minus log ED_{50}.

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Homes for Aged in Sweden

In Sweden, special designs for homes for the aged, developed by Swedish architects in a national competition, provide for division of even the largest home into small units in which six or seven residents share a living room, family-sized dining room, and small kitchen for making coffee and between-meal snacks. Private sleeping and toilet rooms adjoin these common facilities. The casual and informal atmosphere of the architecture is carried over into management of the homes, where there are no rules, regulations, or specified hours for visiting or other activities and no segregation by sexes. The directors (an increasing number of homes are managed by women with 3 years of special training) encourage residents to be as independent as possible. Fees charged by the homes are low enough so that, through pensions and other income sources, all elderly people can afford them.

This Swedish approach to care of the aged is described in an illustrated 32-page booklet, "Homes for the Aged in Sweden Offer Ideas for Americans," prepared by the President's Council on Aging. Issued to meet the growing interest in information about new ways of providing satisfactory care for elderly people who cannot live alone, the publication is available for 60 cents from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., 20402.