Immune Response by the Mouse to Orally Administered Actinophage.* (28462)

S. G. Bradley, Y. B. Kim and D. W. Watson
Department of Microbiology, University of Minnesota, Minneapolis

Orally administered particulate matter and large molecules have been found in the blood, frequently within a few minutes after the initial exposure(1,2). Bacteriophage, for example, was detected in the blood of 85% of mice given *B. megatherium* phage by gastric lavage and 25% of rats given coliphage T1 (2,3). It was estimated that as many as 8×10^4 phage might be present in the total blood volume of a mouse. Because minute amounts of actinophage administered intraperitoneally to the mouse(4) and coliphage to the guinea pig(5) stimulate production of antibody, sera from mice fed actinophage might well be expected to display increased phage neutralizing activity. Indeed, actinophage added to the drinking water appeared in the blood minutes after presentation; after several weeks of continued exposure, sera from the mice displayed appreciable neutralizing activity.

Methods. Adult BALB mice of both sexes were given drinking water containing 10^{10} actinophage MSP8/ml; liquid was withdrawn at night and provided *ad libitum* during the day (6-8 hours). Both crude lysates and purified phage preparations(6) were used to infect the drinking water. Blood samples were collected from the retro-orbital plexus and assayed directly for presence of phage(4); serum was harvested by centrifugation, heat-inactivated and tested for neutralizing activity, *i.e.*, the number of viable phage remaining after incubating the phage-serum mixture for 30, 90, 180 and 360 minutes was determined(7) and activity was expressed as K values(8). To characterize partially the nature of the neutralizing activity, the serum was fractionated on a DEAE cellulose column(9).

Results. Mouse sera collected 30 minutes after administering actinophage orally contained 2×10^3 to 9×10^3 viable phage/ml. Repeated exposure to phage did not decrease phage uptake (Table I). Sera of over 250 normal animals have been checked for appearance of phage in the blood within one day following oral presentation; all have been positive. Blood of mice exposed once to virus-contaminated drinking water contained viable phage for as long as 5 days. Animals given phage orally for many days needed more than 15 days to free their blood of viable phage (Table II). The spleen of mice which drank phage infected water for several weeks did not contain large amounts of phage. Immunization of mice by 1 and 2 intraperitoneal injections of 10^{10} actinophage did not alter phage uptake but did accelerate phage clearance. Blood of mice made hyperimmune as a result of 4 injections of phage did not contain viable phage after oral administration.

After 2 or more weeks exposure to actinophage, the mouse developed substantial neutralizing activity (Table III). Sera from mice fed phage for 4-6 weeks were subjected to chromatographic analysis on a DEAE cellulose column; phage neutralizing activity was separated into 2 fractions, one eluted with pH 6.3 0.015 M phosphate buffer and the other eluted with pH 5.5 0.3 M phosphate buffer (Fig. 1). The neutralizing activity in normal serum and sera of mice fed phage for 2 weeks was found in the pH 5.5 0.3 M phosphate buffer fraction (Fig. 2 and 3). Im-

TABLE I. Phage Content of Blood 3 Hours After Oral Presentation.

Days in exp	Days phage given	No. phage/ml*	No. mice tested
1	1	4.3×10^3	27
2	2	3.0×10^4	6
3	3	1.9×10^4	2
8	6	6.0×10^4	10
15	11	1.0×10^4	15
22	16	2.3×10^4	12
37	26	2.4×10^4	4
48	36	1.0×10^4	10

* Median value.

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TABLE II. Clearance of Viable Phage from Blood After Oral Administration.

Days in exp	Days fed phage	No. phage/ml blood after withdrawing phage: Day 0	Day 1	Day 2	Day 3	Day 4	Day 6	Day 15
1	1	6×10^3	4×10^3	1×10^3	6×10^2	2×10^2	$<10^2$	$<10^2$
15	11	1.6×10^4	9×10^3	3×10^3	1×10^3	3×10^3	2×10^3	4×10^2
35	26	1×10^4	6×10^3	1×10^3	2×10^3	2×10^3	1×10^3	5×10^2
7*	1	6×10^3	7×10^2	3×10^2	1×10^2	$<10^2$	$<10^2$	$<10^2$
14†	1	4×10^3	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
		9×10^2§						
30‡	1	$<10^2$§	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$

* 1 IP injection of 10^{10} phage. † 2 IP injections of 10^{10} phage. ‡ 4 IP injections of 10^{10} phage. § 3 hr after withdrawing phage.

TABLE III. Enhanced Response Elicited by Injection of Antigen into Mice Previously Fed Phage.

1st stimulation	Duration (wk)	2nd stimulation	Bled after (wk)	Neutralizing activity (K value)*
None	2	None	1	.004
Oral daily	2	"	1	.09
" "	2	1 IP	1	23 (87†)
None	2	1 IP	1	1.2
1 IP	1	None	2	24
1 IP	1	Oral daily	2	22

* Sera harvested 21 days after start of experiment.
† K value of sera taken 21 days after IP injection of phage.

munoelectrophoretic analysis and sucrose gradient ultracentrifugation studies of these DEAE fractions have established that the fraction eluted at pH 6.3 by 0.015 M phosphate buffer is 7S globulin and the fraction eluted at pH 5.5 by 0.3 M phosphate buffer is 19S globulin(9).

Mice fed phage for 2-6 weeks developed

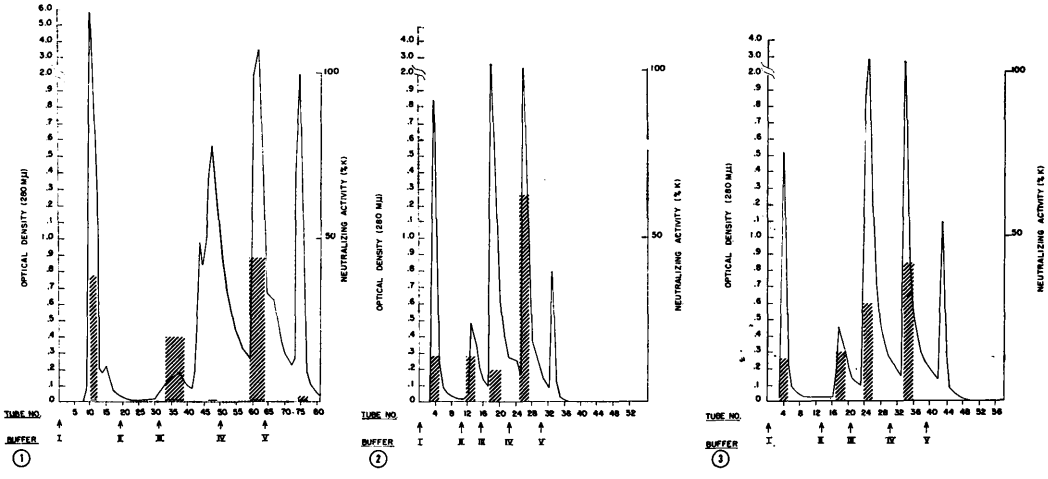

FIG. 1. DEAE chromatographic fractionation of serum from mice fed phage for 4-6 wk. Continuous line shows protein content of various fractions; bars show distribution of phage neutralizing activity among fractions. K value of the whole serum was 0.12.

FIG. 2. DEAE chromatographic fractionation of serum from mice fed phage for 2 wk. Continuous line shows protein content of various fractions; bars show distribution of phage neutralizing activity among fractions. K value of whole serum was 0.13.

FIG. 3. DEAE chromatographic fractionation of serum from normal mice. Continuous line shows protein content of various fractions; bars show distribution of phage neutralizing activity among fractions. K value of the whole serum was 0.003.

20- to 100-fold more neutralizing activity than normal animals. Continued oral administration, however, did not result in increased antibody production. In no experiment did the immune response elicited by continuous feeding of phage equal that obtained by a single injection of 10^{10} phage. Mice previously fed phage daily for 2 weeks, when given antigen intraperitoneally, produced more antibody than mice not fed phage (Table III). Mice stimulated by a single injection of antigen and 7 days later given phage daily for 2 weeks possessed no more neutralizing activity than comparable animals not fed phage. Oral administration of antigen did not elicit an anamnestic response. This experiment did demonstrate that maximal antibody titer was not achieved until 20 days after injection of antigen.

Discussion. Both soluble antigen such as bovine serum albumin(10) and particulate antigens such as actinophage when present in the diet can enter the body proper and elicit antibody production. Prior immunization by 2 injections of antigen and by antibody invoked by oral administration of antigen did not decrease the amount of phage taken in by the mouse. Prior immunization by intraperitoneal injection of antigen increased the clearance rate but prolonged oral administration did not, probably because phage sequestered in the body (but not in the spleen) were continually released for several days. Failure to find phage in the blood of hyperimmunized animals may be attributed to rapid neutralization of phage in the blood or failure of phage to be taken up. These experiments did not differentiate between these alternatives. Based on DEAE elution characteristics, both 7S and 19S globulins are produced but the 19S component prevails initially. Clearly, the early 19S response which gives way to 7S antibody is not dependent upon route of antigen presentation.

In view of the ubiquity of antigens in nature, it is not surprising that normal animals possess small amounts of antibody specific for many diverse antigens. Study of the primary response to antigens such as bacteriophages, bacteria, red cell stroma and serum proteins in normal adult laboratory animals therefore is probably impossible in view of their repeated exposure by the gastro-intertinal route.

Summary. Actinophage added to the drinking water of adult BALB mice appeared in the blood within minutes; continued oral exposure to phage elicited production of phage neutralizing antibody. The initial immune response was 19S globulin; later both 7S and 19S globulins were present. Injection of antigen into mice previously fed phage elicited an enhanced response, but oral administration of phage to mice previously immunized by one injection of antigen did not cause an increase in antibody titer.

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