while cardiac muscle of these immature birds contained almost one-half the adult amount 2 days after hatching, and reached nearly adult levels at 8 days after hatching.

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Antigenicity of Mycobacteriophages R1, D29, and Leo in Rabbits* (33033)

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Among the reports in the literature on mycobacteriophages, there are no systematic studies on the antigenicity of these phages. There are a few reports such as those of Bowman (1), Takeya *et al.* (2), and Mankiewicz (3, 4) which indicate that mycobacteriophages are relatively poor antigens as compared to the antigenicity of other bacteriophages (5). The data in the present paper on the serology of three mycobacteriophages support the concept that mycobacteriophages are poor antigens.

Materials and Methods. Media. Nutrient broth was used for growing the bacteria. Bottom-agar and soft-agar were the same as reported previously (6) and these were used in standard phage assay techniques.

Mycobacteriophages.[2] Lysates of mycobacteria infected with mycobacteriophages R1, D29, or Leo were prepared either on plates or in broth by the techniques of Bowman (6). The bacteria and bacterial debris were centrifuged out of the lysates at 5000*g* for 1 hour. The phages were then pelleted by high speed (25,000*g* for 6 hours or 100,000*g* for 30 min) centrifugation and resuspended in fresh broth. For further purification these suspensions were usually subjected to two or three cycles of high and low speed centrifugations. Such purified phage, suspended in broth, were used as the antigenic stimuli.

Bacteria.[2] *Mycobacterium tuberculosis.* ATCC No. 607 (henceforth referred to as *M.* 607) was used for growing and assaying the phages. *M. butyricum* (R1) was used as the initial source of mycobacteriophage R1.

Production of antiphage antibodies in rabbits. Each of the mycobacteriophages was injected, at weekly intervals, into rabbits for 12 weeks. Two rabbits each were injected with 1.0 or 0.5 ml of each phage by the following three routes: intravenous, subcutaneous in broth, and subcutaneous with incomplete Freund's adjuvant (7). The concentrations of phage used were between 10^{10} and 10^{11} plaque-forming units/ml (pfu). Blood samples were taken from each rabbit

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by cardiac puncture at weekly intervals (except the first week following the initial injection). The blood was collected in sterile tubes and allowed to clot at room temperature. The clots were then ringed and the tubes were stored overnight at 4°C. The following day the clotted blood was centrifuged at 1000*g* for 15–30 min and the sera were collected and stored at 20°C in sealed tubes for subsequent testing.

Neutralization experiments. The stock phage was diluted in broth to a concentration 10 times greater than that to be used in the phage–antiphage reaction mixture (usually about 5×10^7 pfu/ml). The rabbit serum was diluted in broth to the concentration to be used in the neutralization reaction mixture and prewarmed at 37°C. One-tenth ml of the diluted phage preparation was then added to 0.9 ml of the prewarmed antibody solution and the mixture was incubated at 37°C. Then at intervals, the concentration of unneutralized phage in the reaction mixture was determined by removing a sample, diluting it 1:50 or 1:100 to stop neutralization, diluting further if necessary, and plating a sample from the last dilution of the mixture in duplicate or quadruplicate with *M.* 607 in soft agar. A control neutralization mixture consisted of 0.1 ml of the same diluted stock phage mixed with 0.9 ml of broth and incubated at 37°C. Two 0.1-ml samples were removed from the control neutralization mixture immediately after preparation and each was diluted and plated in quadruplicate. This assay process of the control was repeated at the end of each experiment and the mean plaque count from the 16 plates was used to determine the input phage (P_0) concentration. The relative neutralizing activity of each serum (antibody concentration) was calculated in terms of their K values. The later were determined by use of the formula

$$K = 2.3\, D/t \times \log P_0/P$$

where D represents the final dilution of serum in the phage–serum mixture, t is time, and P is the surviving phage concentration at time t. Thus the K value of a given serum is an estimate of its relative antibody content.

Results. Although each experiment in animals was performed in duplicate, only the data obtained from one animal of each pair are reported since there was no great variation between the animals in duplicate experiments.

Antibody production to mycobacteriophage R1. The rate and extent of neutralizing capacity (antibody production) of sera from rabbits injected with mycobacteriophage R1 at weekly intervals by different routes and methods are shown in Fig. 1. In general, it can be seen that the K values of sera obtained from the rabbits inoculated intravenously were higher than respective K values of sera obtained from the rabbits injected subcutaneously. The amount of antibody produced to mycobacteriophage R1 is seen not to be appreciably greater when given subcutaneously with incomplete Freund's adjuvant than when given subcutaneously without adjuvant. In each of the three experiments reported the maximum K values were obtained at about

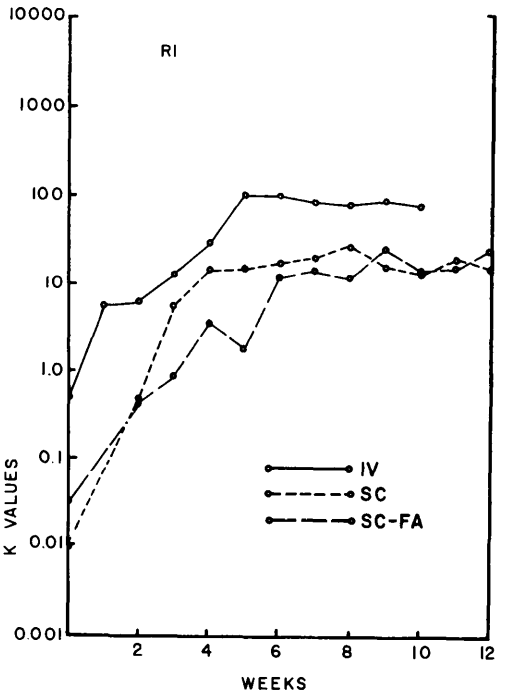

FIG. 1. Increase in K values of sera from rabbits injected with purified mycobacteriophage R1 at weekly intervals for 12 weeks. IV = intravenously; SC = subcutaneously; SC-FA = subcutaneously with incomplete Freund's adjuvant.

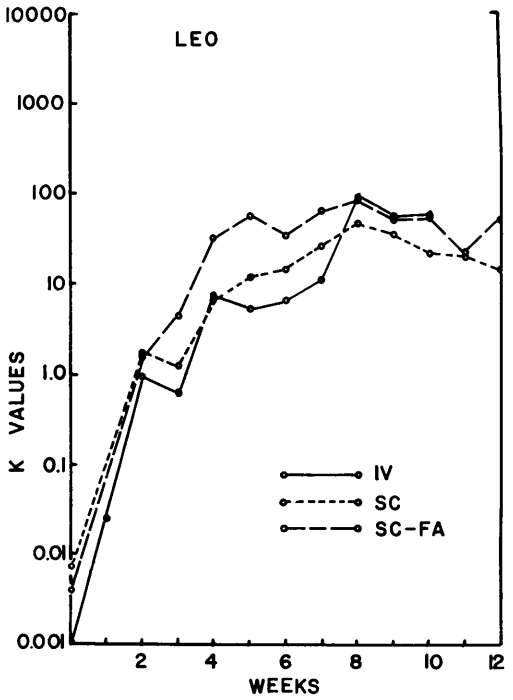

FIG. 2. Increase in K values of sera from rabbits injected with purified mycobacteriophage Leo at weekly intervals for 12 weeks. IV = intravenously; SC = subcutaneously; SC-FA = subcutaneously with incomplete Freund's adjuvant.

the fifth and sixth weeks after the first injection of phage. Subsequently, there was no appreciable change in neutralizing activity of the sera. Sera obtained from the rabbit receiving phages intravenously had maximum K values of about 100, whereas sera obtained from rabbits receiving phage subcutaneously had a maximum K value of about 15–20.

Antibody production to mycobacteriophage Leo. The rate and extent of antibody production in rabbits injected with mycobacteriophage Leo at weekly intervals by different routes and methods are shown in Fig. 2. In general, it is seen that respective K values of serum from each of the three rabbits were very similar over the course of the experiment. In particular, the neutralization activity of the sera of each animal increased almost exponentially for the first 5 weeks of phage injection and after this there occurred a decrease in the rate of antibody production. Peak K values of the sera were obtained from each animal at about the eighth week after the first injection of phage. Subsequently, there was a decline in the activity of the sera.

Antibody production to mycobacteriophage D29. The rate and extent of antibody production in rabbits injected with mycobacteriophage D29 at weekly intervals by different routes and methods are shown in Fig 3. It is seen that the initial rate of antibody production is approximately the same in the three animals. The serum of the rabbit receiving phage intravenously reached its maximum K value at about the sixth week of antigenic stimulation. Subsequently, no significant change occurred in its K values. The serum of the rabbit receiving phage subcutaneously reached its peak K value at the fourth week, and in general, declined slightly during the remainder of the course of antigenic stimulation. In contrast to the two previous animals, the serum of the rabbit receiving phage subcutaneously with incomplete Freund's ad-

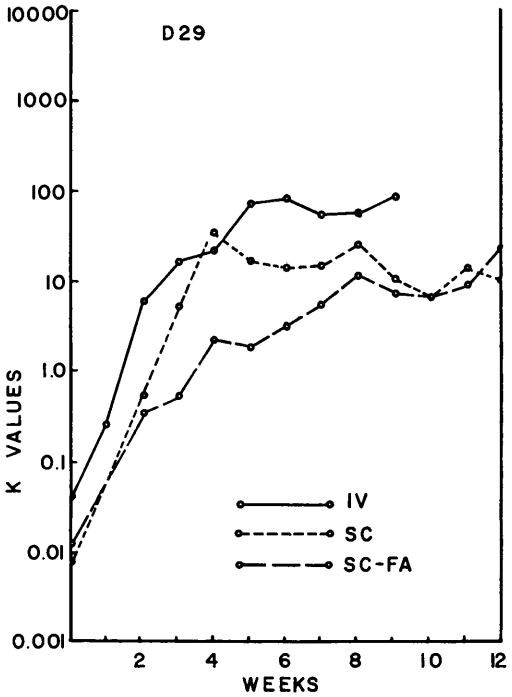

FIG. 3. Increase in K values of sera from rabbits injected with purified mycobacteriophage D29 at weekly intervals for 12 weeks. IV = intravenously; SC = subcutaneously; SC-FA = subcutaneously with incomplete Freund's adjuvant.

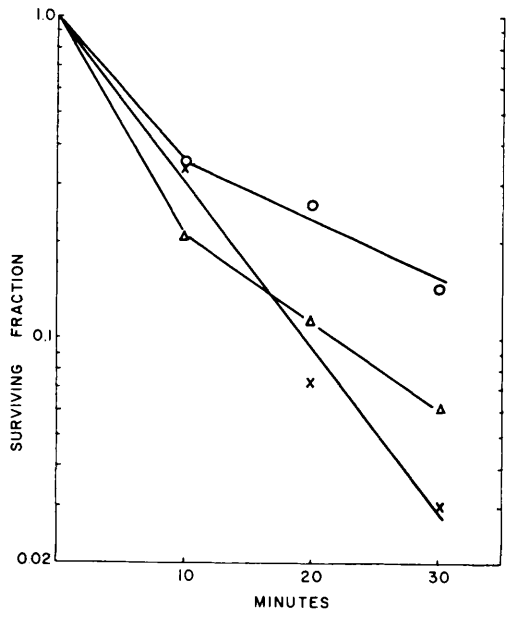

FIG. 4. Neutralization of mycobacteriophages. (○), Leo and anti-Leo tenth-week serum used diluted 1:200, $P_0 = 1.4 \times 10^6$ plaque-formers/ml; (△) R1 and anti-R1 tenth-week serum used diluted 1:1000, $P_0 = 5.7 \times 10^6$ plaque-formers/ml; (×) D29 and anti-D29 ninth-week serum used diluted 1:1000, $P_0 = 8.4 \times 10^6$ plaque-formers/ml.

juvant showed an essentially exponential rate of antibody formation during the first 4 weeks of stimulation. This was followed by a gradual decline in activity, resulting in a nonexponential increase in antibody formation. The greatest amount of neutralizing activity of the serum of this rabbit was obtained at the twelfth week. Thus the sera of the rabbits injected subcutaneously without incomplete Freund's adjuvant or intravenously reached peak K values at the fourth and twelfth week, respectively. It can be seen (Fig. 3) that respective K values were greater in the sera of the rabbits injected intravenously than in rabbits injected subcutaneously with and without adjuvant (except for fourth week sera).

Kinetics of neutralization. The kinetics of neutralization of mycobacteriophages R1, Leo, and D29 by their respective, homologous antiserum are shown in Fig. 4. It is seen that the D29 phage–antiphage system was exponential for the first 97% of the phages neutralized. The kinetics for the Leo and R1 phage–antiphage systems were not exponential. For the Leo and R1 phage–antiphage systems, the rates of neutralization for each system appeared to decrease significantly after the first 10 min of incubation. Subsequently the neutralization rates for each did not change.

Cross neutralization. Mycobacteriophages D29, R1, and Leo were tested for their ability to be neutralized by each of the two heterologous antisera. The sera used were obtained from rabbits injected with phage intravenously. Table I gives the results of a typical experiment; it shows that the K values for each heterologous phage–antigen system was at least 100-fold less than the K value for the respective homologous phage–antiphage system.

Discussion. The data given in Figs. 1–3 clearly indicate that these mycobacteriophages are antigenic. However, they are not nearly as antigenic as *Escherichia coli* phage ϕX174 (5) since the latter evokes K values of three to four thousand after only two or three intravenous injections The present data in conjunction with previous reports (1, 2) and the conclusions of Redmond (8) permit the generalization that mycobacteriophages have limited antigenic capacity. For the three phages studied, the rate of antibody production (increase in K values) was not dependent on the route or condition of injection. The extent of antibody production was greater for phages R1 and D29 when they were injected intravenously. This dependency was not seen with phage Leo.

TABLE I. The K Values of Sera in Homologous and Heterologous Mycobacteriophage Antiphage Systems.[a]

	Phage		
Antiserum to phage	D29	Leo	R1
D29	56.0	0.14	0.31
Leo	0.47	55.2	0.65
R1	0.03	0.0	68.5

[a] $P_0 = 5 \times 10^6$ pfu/ml for each system; each serum used diluted 1:50 in broth; each mixture incubated 30 min at 37°C.

Only the D29 phage–antiphage system was found to have exponential neutralization kinetics. The reason for this is not clear. Cross neutralization experiments have shown that these three phage–antiphage systems are specific and that no cross neutralization occurs between heterologous phage–antiphage systems.

Summary. The antigenicity of several known mycobacteriophages was determined in rabbits. The concentration of antibody production was low (K values 100 or less). The kinetics of neutralization showed that the D29, and not the Leo or R1, phage–antiphage system was exponential. The amounts, but not the rates, of antibody produced were dependent on the route used to inject the animals. No cross neutralization occurred between the heterologous phage–antiphage systems.

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Hepatic Drug Metabolism after Phenobarbital or 3-Methylcholanthrene Pretreatment of Rats Bearing a Pituitary Tumor (33034)

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A decrease in the microsomal metabolism of eight exogenous compounds by liver from rats bearing a somatotropin, corticotropin, and prolactin secreting pituitary tumor (MtT) (1) has been reported (2,3). The decreased metabolism of hexobarbital and aminopyrine by liver from MtT-bearing rats as compared with that of control rats was not mediated via the adrenals or testes, nor were any inhibitors of the *in vitro* hepatic metabolism of these two compounds demonstrated in the liver of MtT rats (4). In the present study, phenobarbital or 3-methylcholanthrene (3-MC) was administered to control and to MtT-bearing rats in order to (a) determine if liver microsomal drug-metabolizing enzyme activity (as measured by the metabolism of hexobarbital, aminopyrine, zoxazolamine, and benzpyrene) was increased after such treatment, and (b) investigate further the type of inhibition of this activity produced by growth of the MtT.

Materials and Methods. Male Fischer rats served as donors and recipients of the MtT [Furth strain MtT-F4 (5)]. After excision from donor animals, the tumor was homogenized in 0.9% aqueous saline with a glass homogenizer. The forty-first and forty-second generations of MtT were used, and at the time of autopsy, morphologic evidence for the secretion of corticotropin, prolactin, and somatotropin by the tumor was noted as previously described (3). Rats were given food and water *ad libitum.*

Rat livers were excised, and 1 gm of liver was homogenized with 2 ml of 1.15% KCl in a glass homogenizer which had a teflon pestle. The liver homogenate was centrifuged at 9000*g* at 4°C for 20 min. The liver 9000*g* supernatant fraction (0.25 ml equivalent to 1/12 gm of liver) was added to an incubation mixture which contained (as μmoles/2.5 ml of the mixture) glucose-6-phosphate, 12.5; $MgSO_4$, 12.5; and nicotinamide adeninedinucleotide phosphate (NADP), 2.08. The μmoles of substrate per 2.5 ml of reaction mixture