ANTIBODY FORMATION*

III. The Primary and Secondary Antibody Response to Bacteriophage ϕX 174 in Guinea Pigs

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Quantitative studies of antibody formation in experimental animals have been facilitated by the use of labeled proteins (1, 2). After equilibration following intravenous injection, labeled antigen is eliminated from the circulation in two exponential phases: the first and slower rate is due to non-immune catabolism; the second and rapid decline is caused by the production and release into the circulation of specific antibody (3, 4).

In this study, guinea pigs have been injected with relatively minute amounts of a small bacteriophage, ϕX 174, which has been traced in the circulation utilizing the plaque-forming capacity of the virus. With this method it has been possible to detect an immune elimination as early as 24 hours after injection. The early detection of antibody was facilitated by the small amounts of antigen employed. It has also been shown that there is a close resemblance between the kinetics of the primary and secondary antibody response to this bacteriophage.

Materials and Methods

Phage.—Strains of ϕX 174 and T_2 were kindly supplied to us by Dr. E. Lennox. ϕX was grown in *Escherichia coli* strain C in glycerol-casamino acid medium (5). The lysed culture was centrifuged at low speed to remove cell debris. Purification was then accomplished by precipitation with ammonium sulfate, passage through a DEAE cellulose anion exchange column, and elution with 0.1 M ammonium acetate (6). This purified stock containing 10^{11} plaque-forming particles per ml was kept at 4°C in ammonium acetate buffer pH 7.4 containing 0.01 per cent gelatin. Dilutions of this stock were used for almost all the immunization experiments.

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T_2 phage grown in *E. coli* B 1, 5 and purified by differential centrifugation (7) contained 10^{13} plaque-forming particles per ml.

In this paper, the number of phage particles will refer only to the plaque formers.

Immunization.—Dilutions of phage were injected intravenously into the hind leg of 400 gm Hartley guinea pigs. For tracing phage in the circulation, quantitative bleedings of 0.1 to 1 ml were performed from the retro-orbital space using a capillary pipette rinsed with heparin. The blood was immediately placed in 0.9 ml chilled heparinized saline, or for low titers of phage, was not diluted and was kept at 4°C until assayed. The number of plaque-forming particles in whole blood was determined using strain C of *E. coli* for the assay utilizing the pour plate technique of Adams (8). For serum antibody studies, approximately 1 ml of blood was obtained and allowed to clot.

Antibody Titrations.—The assay for antibody was carried out by the method described by Adams (7). The inactivation of phage by antibody follows first order kinetics which is described by the relationship:

$$\ln \frac{P_t}{P_0} = \frac{Kt}{D}.$$

P_0 is the phage assay at zero time; P_t is the phage assay at time t minutes; D is the final dilution of antiserum; and K minutes^{-1} is the first order inactivation constant. K, which is independent of dilution, is considered to be a measure of antibody with two important reservations: (*a*) ϕX, like T_2, may stimulate the production of antibody molecules which differ in their capacity to neutralize the infectivity of phage (9). Unlike T_2, however, ϕX is a small virus, which is symmetrical and simpler in physical structure closely resembling a dodecahedron (6). (*b*) Antibody that is highly dissociable will not be measured.

In practice, we found that neutralization of ϕX frequently did not follow first order kinetics and was not reproducible unless strict attention was paid to the following factors: (*a*) Phage preparations whose titers had dropped more than 25 per cent were not used. (*b*) Not all phage preparations were equally satisfactory. It was necessary for us to screen 4 different preparations of ϕX in order to find one, obtained through the courtesy of Dr. I. Tessman, which was suitable. (*c*) The phage preparation was incubated at 37°C for 30 minutes prior to the assay for antibody. (*d*) 10 per cent normal rabbit or beef serum in sterile saline was used as diluent in order to stop the neutralization reaction at the indicated time. (*e*) The extent of neutralization of phage by antiserum was determined at 4 or more points in time and the phage-serum mixtures were always double plated. (*f*) The conditions of titration were varied. For example, in some instances, phage neutralization was examined over a 2 log fall in phage titer for 15 minutes and using a higher dilution of antiserum, over a 1 log decline in phage titer for 1 hour in order to compare the rates of inactivation that were obtained. Despite attention to these factors, some sera, particularly from primarily immunized animals did not show exponential neutralization over a period of one hour. Since this finding appeared to be due partly to dissociation of antibody from initially neutralized virus, the early phase of neutralization was used in these instances.

Rabbit anti-guinea pig γ-globulin was absorbed with the non-γ-globulin fractions of guinea pig serum obtained by starch electrophoresis (10). This antiserum contained 2.0 mg of antibody protein and gave a single line of precipitation with whole guinea pig serum or guinea pig γ-globulin by immunoelectrophoresis (11).

X-Irradiation.—Whole body x-irradiation was administered to guinea pigs by a 220 kv Picker x-Ray Machine. Irradiation was through the ventral surface of the animal at a distance of 50 cm from the tube. With the machine operating at 220 kv and 20 milliamps and using a filter of 0.5 millimeter copper and 1 millimeter aluminum, 40 r per minute were delivered as a midphantom dose as measured by a Victoreen ionization meter on a revolving platform.

RESULTS

Elimination of ϕX from the Circulation of Guinea Pigs.—The elimination of 10^9 ϕX from the blood of a 400 gm guinea pig is shown in Fig. 1. Unlike heterologous serum proteins, the phage did not pass into the extravascular compartment, but declined exponentially at the rate of approximately 1 log per day for 48 hours. At this time, the rate of elimination markedly increased and detectable phage disappeared from the circulation within the following 24 hours. In additional similar experiments, phage was traced in the serum, and also in whole blood after freezing and thawing five times to disrupt leucocytes. These

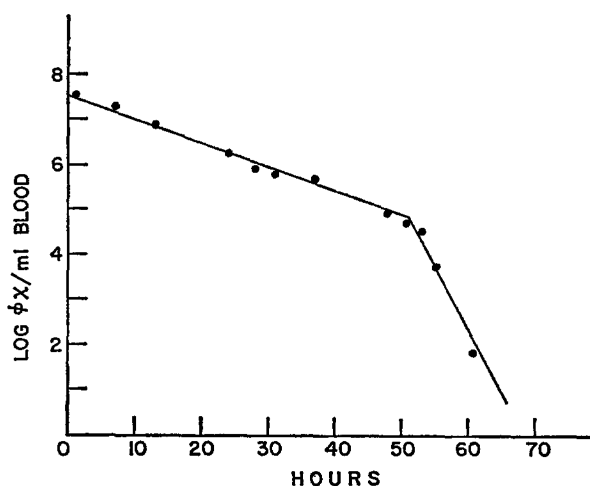

FIG. 1. Elimination of 10^9 ϕX from the circulation of a 400 gm guinea pig.

studies yielded analogous results to the first experiment; hence in all further phage elimination studies, whole blood was used because of convenience.

The accelerated elimination of phage appeared similar to the immune elimination of heterologous serum proteins. To prove the immune nature of accelerated phage elimination, additional experiments were performed.

To test for the specificity of accelerated elimination, two guinea pigs were injected with 10^9 T_2 phage 48 hours before 6×10^8 ϕX. The pretreatment with T_2 did not affect the time of onset of the accelerated elimination of ϕX. In contrast, injection of 6×10^8 ϕX in two guinea pigs 48 hours before a second injection of 6×10^8 ϕX resulted in an immediate accelerated elimination of the latter.

In order to test for antibody after elimination of phage, serum was obtained from three guinea pigs 72 hours after injection of 6×10^8 ϕX. Incubation of this serum with 10^6 ϕX resulted in partial neutralization of the ϕX. Sera obtained from these guinea pigs during the following weeks showed an increased

neutralizing capacity. As shown in Table I, this neutralizing factor for ϕX caused no neutralization of T_2 bacteriophage, was stable to heating at 56°C for 30 minutes, and was no longer demonstrable in the serum after preincubation with rabbit anti-guinea pig gamma globulin. Thus, the neutralizing factor satisfies the criteria for antibody: it appeared after immunization, was specific for the antigen, and was associated with the gamma globulin fraction of the serum.

TABLE I

Specificity and Nature of the Neutralizing Factor

Media*	No. of plaques	
	ϕX	T_2
Buffer	103	98
Unheated anti-ϕX serum‡	2	92
Heated anti-ϕX serum (56°C for 30 min.)	1	101
Anti-ϕX serum + rabbit anti-guinea pig gamma-globulin§	100	—
Anti-ϕX serum + rabbit anti-BSA + BSA‖	56	—

— = not done.

BSA = bovine serum albumin.

* 1.2 ml of media and 0.1 ml of ϕX or T_2 (10^6/ml) were incubated at 37°C for 1 hour before a 10^{-3} dilution was plated.

‡ A 1:13 dilution of pooled serum obtained 1 month after primary immunization with 6×10^8 ϕX.

§ 1 ml of rabbit antiserum containing 2 mg of anti-guinea pig gamma-globulin was incubated with the anti-ϕX serum at 37°C for 30 minutes and kept at 4°C for 48 hours. After centrifugation, 0.1 ml of supernatant was used.

‖ To exclude a non-specific effect of specific precipitation on phage, 100 μg of BSA and excess rabbit anti-BSA were successively added. An unexplained partial neutralization of phage resulted. Appropriate control tubes containing either BSA, rabbit anti-BSA, or rabbit anti-guinea pig gamma-globulin did not show neutralization.

It is known that a sufficient dose of ionizing radiation administered before injection of antigen can prevent a detectable antibody response (12). Three guinea pigs received 400 r whole body x-irradiation 48 hours before they and one unirradiated animal were injected with 6×10^8 ϕX. As shown in Fig. 2, prior irradiation prevented the accelerated elimination of ϕX.

These experiments indicate, therefore, that the accelerated elimination of ϕX from the circulation of guinea pigs, like the immune elimination of heterologous serum proteins, can be considered to be caused by the formation and release into the circulation of specific antibody.

Is This a Primary Antibody Response?—The early antibody response to ϕX suggested the possibility that the guinea pigs used in our experiments had had

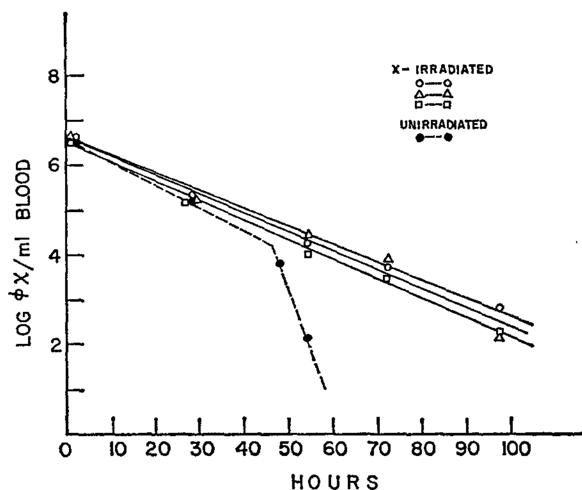

FIG. 2. Elimination of 6×10^8 ϕX from the circulation of 1 unirradiated guinea pig and 3 animals that received 400 r whole body x-irradiation 48 hours previously.

TABLE II

The Neutralizing Activity of Sera from Non-immunized Guinea Pigs

Media	Tube No.	K§ $\times 10^{-4}$
Adult guinea pig sera*	1	5.0
	2	8.0
	3	11.0
	4	8.5
	5	8.9
	6	9.4
	7	8.3
	8	10.0
Neonatal guinea pig sera‡	9	18.0
	10	10.0
	11	5.8
0.1 per cent gelatin in saline with 0.001 M $CaCl_2$	12	35.0
	13	30.0
	14	35.0
10 per cent bovine serum albumin in saline	15	33.0
1 per cent human serum albumin in saline	16	14.0

* Obtained from 8 non-immunized animals weighing 350 to 450 gm.

‡ Obtained from 3 neonatals bled within 1 hour after birth.

§ Samples were incubated for 52 hours.

previous antigenic stimulation with ϕX by contact with an infected bacterial strain. In order to investigate this possibility, we examined serum from non-immunized animals for the presence of antibody, attempted to immunize neonatals that have been protected from prior antigenic exposure to ϕX, and compared the antibody responses following a first and second injection of ϕX.

The neutralizing capacity of 8 sera obtained from non-immunized adult guinea pigs, 3 sera obtained fom neonatal guinea pigs, a gelatin-saline solution and bovine and human serum albumin solutions were studied. As can be seen

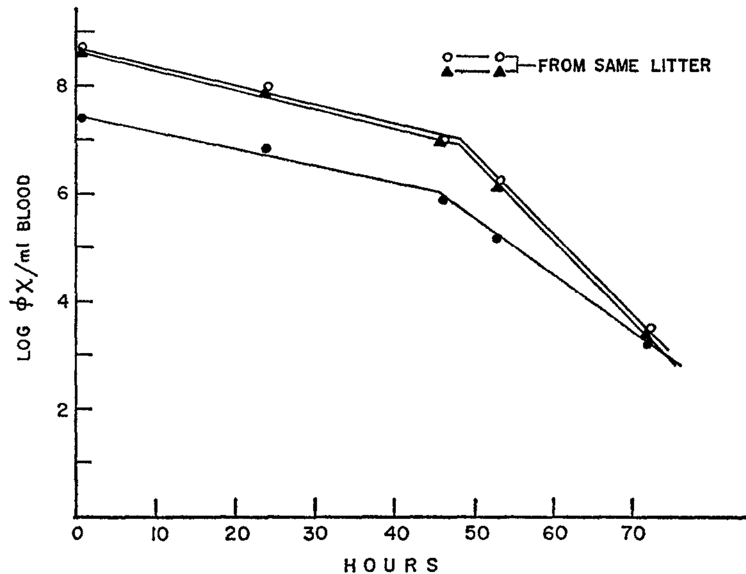

FIG. 3. Elimination of 6×10^8 ϕX from the circulation of 3 newborn guinea pigs less than 3 hours of age.

in Table II, the neutralizing capacity of adult guinea pig sera ranged from 5 to 11×10^{-4}; of neonatal sera from 6 to 18×10^{-4}; and of 0.1 per cent gelatin in saline or 10 per cent bovine or human serum albumin solutions, above 14×10^{-4}. Thus, no evidence was obtained to indicate previous immunization with ϕX in the guinea pigs used throughout this study.

For immunization of newborns, 6×10^8 ϕX were injected into three newborn guinea pigs[1] obtained from 2 litters all less than 3 hours of age. As shown in Fig. 3, immune elimination began in all 3 animals at 44 to 48 hours.

[1] These newborns were selected for immunization because of their heavy birthweight: 120, 118, and 100 gms, since previous studies of sensitization of guinea pig embryos indicated that only the heavier newborns were capable of displaying delayed type hypersensitivity skin reactions (13).

Finally, antibody studies were performed upon sera obtained from guinea pigs after a first and second injection of 6×10^8 ϕX as will be described in detail in another section of this paper. The serum antibody titer one week after a second injection of ϕX was approximately 100-fold higher than the antibody titer one week after a first injection.

These experiments indicate that the immune response to a first injection of ϕX in guinea pigs is usually a primary immunological response.

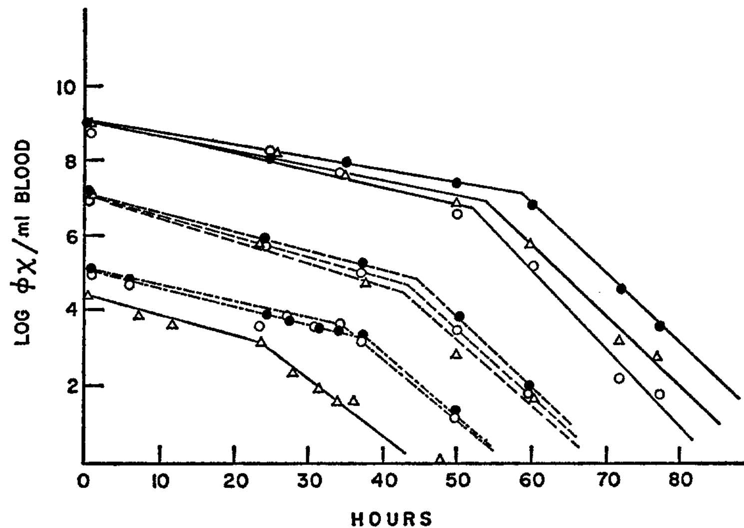

FIG. 4. Effect of dose on phage elimination. Groups of animals were injected intravenously with 6×10^{10}, 6×10^8 or 6×10^6 ϕX from the same phage preparation. The animal whose initial titer was 5×10^4 (solid line) received 3×10^6 ϕX from a different phage preparation.

Effect of Dose of Phage on Time of Onset of Immune Elimination.—The short "latent" period after immunization with ϕX could be related to the relatively small dose employed. In order to study the effect of dose of phage on the time of onset of immune elimination, groups of three guinea pigs were injected intravenously with either 6×10^{10}, 6×10^8 or 3 to 6×10^6 ϕX respectively. As shown in Fig. 4, decreasing the dose of phage decreased the time of onset of detectable immune elimination. It appears that decreasing the dose of phage does not result in a directly proportionately smaller antibody response, although decreasing doses do require proportionately less antibody to result in *detectable* immune elimination. This experiment indicates that the early detection of antibody in our system is due in part to the small amounts of antigen employed.

Kinetics of the Primary and Secondary Antibody Response.—The kinetics of increase in serum antibody was studied after primary and secondary immuniza-

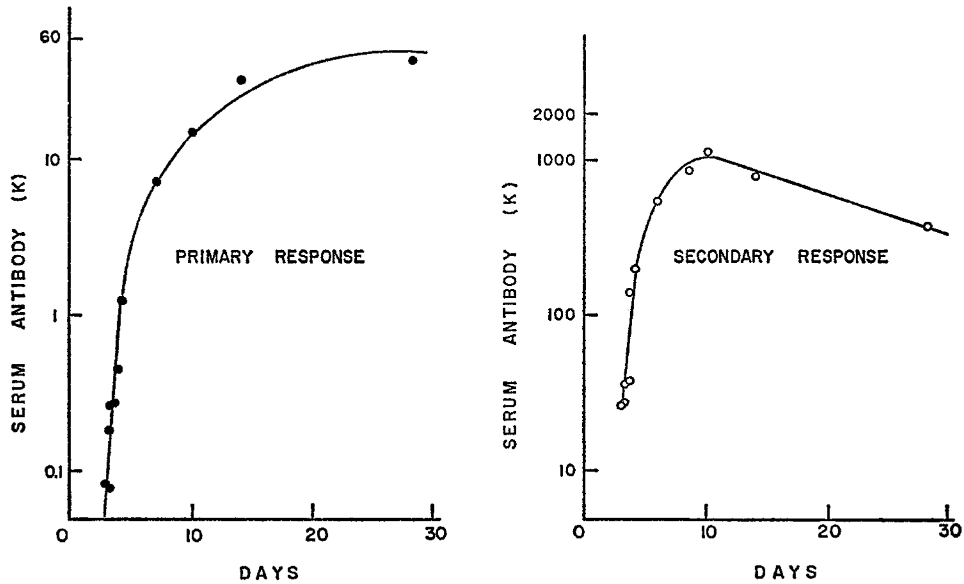

FIG. 5. A representative primary and secondary antibody response to intravenous injection of 6×10^8 ϕX. The interval between injections was 3 weeks for the secondary response.

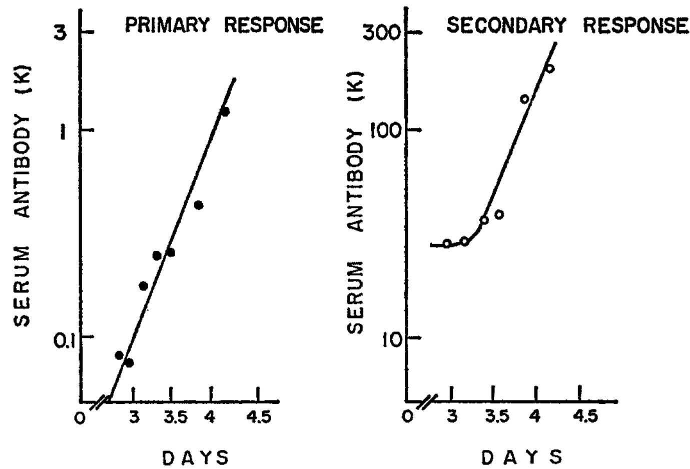

FIG. 6. The early kinetics of a representative primary and secondary antibody response. The animals are the same ones shown in Fig. 5.

tion with ϕX. Nine guinea pigs were immunized with 6×10^8 ϕX intravenously and after 72 hours serum was obtained from 5 of these animals at intervals for a period of several weeks. The remaining 4 animals were rechallenged three weeks later with 6×10^8 ϕX and were similarly bled. The anti-ϕX content (K)

was determined for each serum. The same guinea pigs could not be used for both immunizations, since those animals subjected to repeated retro-orbital bleedings could not be similarly bled again.

Figs. 5 and 6 illustrate a representative primary and secondary antibody response. As can be seen, both responses appeared exponential for approximately 24 to 36 hours after circulating antigen had been eliminated. The length of time for doubling the serum antibody levels of the 5 primarily immunized

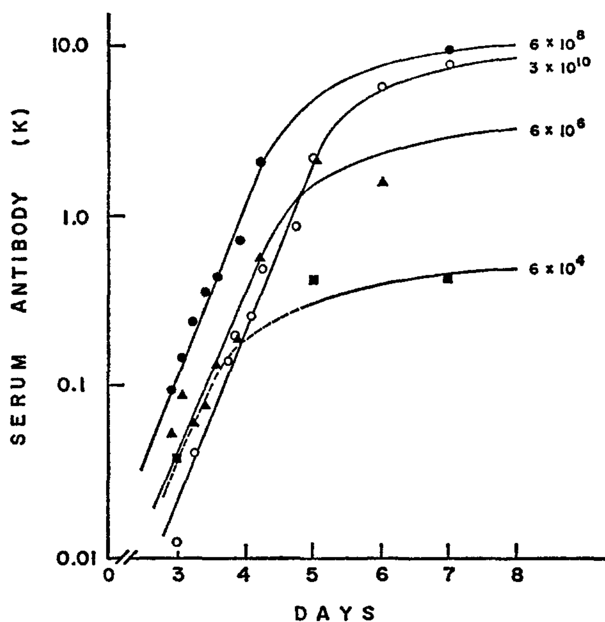

FIG. 7. Effect of dose on the kinetics of antibody formation. The serum antibody response of representative animals injected intravenously with 3×10^{10}, 6×10^{8}, 6×10^{6}, and 6×10^{4} ϕX are shown. Dilutions of the same phage preparation were used except for the 3×10^{10} dose.

animals varied from 6 to 8.5 hours, and for the secondary antibody response 8, 8.5, 8.5, and 15 hours, respectively. In both responses, the exponential phase of antibody synthesis ceased at 4 to 6 days after immunization.[2] Thus, the early kinetics of the primary and secondary response are quite similar, although, as expected, the secondary antibody response titers go up to a K of 10^3 compared to 10^{1-2} after primary immunization.

Figs. 5 and 6 also indicate that there is a longer latent period after secondary

[2] Previous studies have shown that labeled amino acids appear in serum antibody within 40 minutes during active antibody synthesis in rabbits (14). It has also been demonstrated that serum antibody is not stored to any significant degree in the spleen (15). These and other studies suggest that the initial rise in serum antibody is an accurate reflection of antibody synthesis.

challenge compared to primary immunization. Serum antibody levels did not increase until 3 to 4 days after secondary challenge in contrast to primary immunization in which serum antibody can be detected within 24 hours. This finding is probably due to the effect of excess serum antibody present before the second but not the first immunization with ϕX. It is known that excess serum antibody can delay the secondary antibody response in guinea pigs (16).

Fig. 5 also shows that the serum antibody level begins to decline sooner during the secondary antibody response. 7 guinea pigs primarily immunized with either 10^6, 10^8 or 10^{10} ϕX increased or maintained antibody levels during 1 month of observation. In contrast, 2 animals secondarily immunized with 6×10^8 ϕX showed decreasing levels of serum antibody within 2 weeks after immunization.

Kinetics of the Primary Antibody Response after Varying Doses of Antigen.— The kinetics of serum antibody response was determined in groups of 2 to 3 animals after intravenous injection of 10^4, 10^6, 10^8, or 10^{10} phage. The antibody response of a representative animal in each group is shown in Fig. 7. Each animal in which a sufficient number of antibody determinations were made appears to show an exponential phase with a similar rate constant[3] (relative rate) for serum antibody increase. Thus, the relative rate of antibody formation to ϕX is independent of a 10^4 dose range of phage. Fig. 7 shows, however, that the magnitude of the antibody response is dependent on the dose of phage at least in the range of 10^4 to 10^8 ϕX.

The smallest dose employed in our studies was 6×10^2 phage. Intravenous injection of this dose into 10 guinea pigs stimulated a detectable antibody response in 3 of 10 animals, but there was insufficient serum to obtain a precise measurement. It appeared possible that the immune response to this "threshold" dose was an all-or-none phenomenon; therefore, it is important to determine whether or not there is a random distribution of antibody levels in the sera of successfully immunized animals. Unfortunately, we have not yet been able to reproduce this experiment.

DISCUSSION

In the studies reported here a small bacteriophage, ϕX 174, has been injected intravenously into guinea pigs and its elimination from the circulation followed by utilizing the plaque-forming capacity of the virus. The initial phase of the elimination is exponential with a decline of one log per 24 hours. At 24 to 60 hours, depending on the dose of phage injected, an accelerated phase of elimination begins and phage disappears from the circulation within the following 24 hours. The immune nature of the accelerated phase of phage elimination was firmly established by the following observations: (*a*) Specificity of elimination. Injection of T_2 bacteriophage 48 hours before ϕX did not change the time of

[3] The rate constant is the constant k in the exponential form e^{kt} for the exponential rise of serum antibody and represents the slope of that curve plotted on semilogarithmic paper.

onset of accelerated elimination of ϕX. In contrast, injection of ϕX 48 hours previously resulted in an immediate accelerated elimination of the second administration of ϕX. (*b*) A neutralizing factor appeared in the serum after phage elimination and increased in amount over a period of several weeks. The neutralizing factor was specific for ϕX (no detectable neutralization of T_2), was stable to heating at 56°C for 30 minutes, and was no longer demonstrable after treatment of the serum with rabbit anti-serum directed specifically against guinea pig gamma-globulin. Thus, the neutralizing factor satisfies the criteria for antibody. (*c*) 400 r whole body x-irradiation administered 48 hours before immunization prevented the accelerated elimination of phage. Thus, the immune elimination of ϕX appears analogous to that previously described for I^{131} trace-labeled heterologous serum proteins (3). It should be noted, however, that the immune elimination of ϕX is a more complex process than is the case for the latter antigens. Unlike I^{131} labeled proteins, it is possible that plaque-forming ϕX can be neutralized by antibody before it is removed from the circulation as an antigen-antibody complex.

The question immediately arose as to whether or not this early immune response was a primary one or whether our guinea pigs had been previously sensitized to ϕX by contact with an infected bacterial strain. The following findings strongly suggest that the early appearance of antibody following a first injection of ϕX is usually a primary immune response: (*a*) The typical early immune elimination was obtained in three neonatal guinea pigs (from 2 litters) injected within the first three hours after birth with 6×10^8 ϕX. Similarly, 8 consecutive newborn human infants injected within 14 to 84 hours after birth with ϕX all showed high antibody titers one week later (K of 2 to 10.5, or 10^3 above background) (17). (*b*) None of 8 individual sera from non-immunized guinea pigs had detectable antibody. All had K values in the same range as neonatal guinea pig sera (see Table II). (*c*) The antibody titer one week after a second injecion of 6×10^8 ϕX was approximately 100-fold greater than the antibody level one week after a first injection.

Thus, a primary antibody response to ϕX can be detected as early as 24 hours after immunization. (See Fig. 4). Moreover, by extrapolation of the rate of serum antibody increase back to background levels, (10^{-3}) it is apparent that antibody synthesis probably begins before 24 hours, and considering the antibody binding effect of circulating antigen, it may begin immediately after injection of antigen. This finding is in marked contrast to the 6 to 13 days before detection of immune elimination of I^{131} heterologous serum proteins in guinea pigs (18). This striking difference in results can be accounted for in part by the relatively minute quantities of antigen used in the phage system (approximately 10^{-6} of the amount of heterologous serum proteins), since increasing the dose of ϕX was shown to *delay* the time of onset of detectable immune elimination. For example, the times of onset of immune elimination for 3–6 $\times$

10^6, 6×10^8 and 6×10^{10} ϕX were 24 to 40, 40 to 50, and 50 to 60 hours, respectively. These findings suggest that part if not all of the "latent" period seen after immunization with conventional amounts of antigen actually represents formation of relatively small amounts of antibody whose reaction with the administered antigen is not detected. This interpretation does not necessarily indicate that antibody production begins immediately after immunization with a soluble protein antigen. For example, other factors may participate in the early antibody response to ϕX, such as the particulate nature of the phage and its excellent antigenicity. Indeed, as little as 6×10^2 plaque-forming phage (approximately 10^{-8} μg. protein) sufficed to stimulate detectable antibody formation in 3 of 10 animals. There are several reports in the literature of similarly early antibody formation following a first administration of antigen (19–21), however, the specificity (immune nature) of the *early* response and/or the absence of previous antigenic stimulation was usually not satisfactorily established. Of particular interest to our studies is a statement of Jerne (22) that a second dose of T_2 bacteriophage is more rapidly removed from the circulation of rabbits if a first injection had been given at least 48 hours previously. By analogy with our present findings, the cause of the accelerated elimination of T_2 phage in Jerne's experiment was probably specific antibody formation.

Although the initial capacity for antibody production is larger in the secondary compared to the primary antibody response to ϕX, the kinetics of increase in serum antibody levels of both responses are remarkably similar. In each, the initial rise in serum antibody levels appeared exponential until 4 to 5 days after injection of antigen and was followed by a much slower rise in serum antibody which continued for at least 1 week. Moreover, the rate constant (relative rate) of antibody formation[2, 3] was usually similar after both primary and secondary challenge. Of 12 animals studied after primary immunization with ϕX, the length of time for doubling the serum antibody level was 6 to 8.5 hours compared to 8, 8.5, 8.5, and 15 hours for 4 animals studied after secondary challenge. A second point of particular interest is the relationship of dose of phage to the primary antibody response. Over a 10^4 dose range of ϕX, the magnitude of the antibody response was affected, primarily by changes in the duration of the exponential phase of antibody formation. In contrast, the relative rate of antibody formation remained constant during this exponential phase (Fig. 7). The simplest but not unique interpretation of the constancy of the relative rate of antibody formation under these varied immunologic situations is that the factors which determine this rate do not change, namely, the relative rate of antibody production per cell and the relative rate of proliferation of antibody-producing cells. An alternative but unlikely possibility is that both these latter rates may change, but in such a fashion that their product, the relative rate of antibody formation, remains constant. Our tentative interpretation is that the quantitative difference between the primary and secondary

response results from the number of cells producing antibody. A definitive test of this hypothesis, however, will require the determination of the kinetics of antibody formation by single cells isolated during the primary and secondary responses.

The kinetics of antibody formation to ϕX show certain similarities to those obtained by Taliaferro and Taliaferro (15) in their extensive studies of hemolysin formation in rabbits. They found that the primary hemolysin response could usually be represented by a series of discontinuous curves each one described by a different exponential rate. Vicari and Lennox (23) have also obtained exponential and similar rates of antibody formation during the primary and secondary antibody response to bacteriophage T_2 in rabbits. In contrast to results with ϕX, however, the primary antibody response to soluble proteins has generally been considered to be non-exponential (24). One explanation for the differences in the early kinetics of antibody formation may be the extreme sensitivity of the assay for antibody to phage. For example, the exponential phase of the primary antibody response to 6×10^8 ϕX was completed within 5 days after immunization at a time when serum antibody concentrations were still low. This phase, therefore, could easily have escaped detection if the assay for antibody were less sensitive, as for example, the method of quantitative precipitation. On the other hand, the operational differences that exist between immunization with ϕX and heterologous serum proteins may be a result of differences in the biological response to these 2 types of antigens.

Our studies have also revealed 3 differences between the primary and secondary antibody response to ϕX in addition to the magnitude of the response: (*a*) the "latent" period is longer after *secondary* challenge. This paradoxical finding is readily explained by the fact that excess serum antibody was present before the second but not the first injection of phage. Previous studies have shown that excess guinea pig diphtheria antitoxin can delay the secondary antitoxin response in the guinea pig (16). Although the presence of serum antibody at the time of secondary challenge represents an additional difference between primary and secondary immunization, this difference does not affect the immune response if sufficiently large amounts of antigen are used for reimmunization thus providing conditions of antigen excess *in vivo*. In our system, however, the amount of antigen is minute, approximately 0.001 micrograms of protein, so that the antigen-antibody interaction that takes place *in vivo* is undoubtedly far in the zone of antibody excess, (*b*) serum antibody levels appear to fall more rapidly after secondary challenge. None of 7 primarily immunized animals showed declining serum antibody levels during the first month after immunization in contrast to 2 secondarily stimulated animals in which serum antibody levels began to fall within 2 weeks. More secondarily immunized animals will have to be studied, however, before concluding that this difference is significant. (*c*) During the primary but not the secondary

antibody response the physico-chemical properties of the antibody molecules that are produced change (17). During the first two weeks after primary immunization, the population of anti- ϕX molecules in the circulation is predominantely of 19S sedimentation constant. Between 2 to 6 weeks, these molecules are steadily replaced by antibody molecules of 7S type. The antibody molecules produced during the secondary response continue to be of 7S sedimentation constant. Similar observations have been made by Stelos *et al.* (25), and Fink *et al.* (26).

Finally, our findings have implications concerning 2 current problems of antibody formation: (*a*) The relation between delayed type hypersensitivity and antibody formation. The observations of Dienes (27) and Salvin (28) that delayed type hypersensitivity frequently appears several days before serum antibody can be detected has been used to support the suggestion that delayed type hypersensitivity is a stage of antibody formation. The present finding that antibody to phage can be detected as early as 24 hours after immunization highlights the difficulty in interpreting the relationship of 2 immune responses in which the relative sensitivities of the assay systems employed are not known.[4] (*b*) Theories of antibody formation. The "latent" period before the appearance of antibody has been considered in instructive hypotheses to represent the time required for antigen to alter the gamma globulin–synthesizing mechanism of the host (29). This interpretation has been fortified by the constancy of the "latent" period with a particular antigen-host combination under a variety of circumstances such as transfer of varying doses of stimulated cells to an immunologically incompetent recipient (30). Our studies indicate that part if not all of the "latent" period is artifactual, thus invalidating this particular argument for proposing an instructive theory.

SUMMARY

Injection of a small bacteriophage ϕX 174 into guinea pigs results in an accelerated elimination of phage detectable as early as 24 hours after injection. The immune nature of the accelerated elimination is indicated by its specificity, by the appearance of excess specific serum antibody after phage elimination, and by the prevention of accelerated elimination by 400 r whole body x-irradiation of guinea pigs prior to injection of phage. The early antibody response is considered to be a primary one since an analogous response occurs in newborn guinea pigs, antibody is not detectable in the sera of non-immunized animals, and the second challenge with ϕX stimulates a serum antibody response 100-fold greater than that after primary immunization. The early detection of immune elimination appears to be due, in part, to the small amounts of phage

[4] Delayed type skin hypersensitivity specifically directed to ϕX has not yet been demonstrated in guinea pigs immunized with 6×10^8 ϕX, either in saline intravenously or emulsified with complete Freund's adjuvant in the footpad.

employed, since larger doses of phage delay the time of onset of detectable immune elimination. The early rise of serum antibody in the primary and secondary response appears exponential with a similar rate constant of antibody formation. The rate constant is also independent of dose. These findings have led to the suggestion that during this exponential phase, the relative rate of antibody formation at a cellular level may be constant for a given antigen.

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