

Adenovirus Antibody Measured by the Passive Hemagglutination Test

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ABSTRACT

LEFKOWITZ, STANLEY S. (Variety Children's Research Foundation, Miami, Fla.), JULIA A. WILLIAMS, BERNARD E. HOWARD, AND M. MICHAEL SIGEL. Adenovirus antibody measured by the passive hemagglutination test. *J. Bacteriol.* **91**:205-212. 1966.—Rabbits immunized intravenously with adenovirus type 5 antigen were tested for antibody titers by use of the passive hemagglutination test (PHA). Primary and secondary responses were studied, and the class of antibody was determined by means of density gradient centrifugation and reduction with 2-mercaptoethanol (ME). It was found that the PHA was 10 to 100 times more sensitive than complement-fixation and neutralization tests for the detection of antibodies to adenovirus. The immunological response to primary immunization was dependent on the dose of antigen, with antibody appearing in as early as 3 days. After secondary stimulation with the same antigen, there was a rapid response which appeared to be less dose-dependent. It was found that a heavy 19S antibody sensitive to ME was produced early and was followed by a lighter, presumably 7S, ME-resistant antibody. Upon secondary stimulation, both 7S and 19S antibody increased to levels greater than those of the primary injection.

The agglutination of red blood cells (RBC) passively coated with antigen has become one of the most sensitive tools for the study of immunoglobulins. It was reported by Friedman and Bennett (3) and confirmed by Ross and Ginsberg (7) that the passive hemagglutination test (PHA) could be employed for the detection of adenovirus antibody in human sera. These investigators reported that the sensitivity of this method was greater than that obtained by complement fixation (CF) and neutralization tests (NT). Prier and Le Beau (6) found the PHA superior to the CF and conglutinating complement adsorption tests using rabbit antiserum. Other viruses have been studied by use of these techniques, including herpes simplex (8), polio (4), and psittacosis (1).

The present study had three aims: to ascertain the sensitivity of this method for the detection of small amounts of antibody, to evaluate this procedure as a tool for studies of primary and secondary responses of rabbits to virus immunization, and to determine the applicability of the PHA test for assays of antibodies associated with different fractions of immunoglobulin.

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MATERIALS AND METHODS

Virus. For the cell sensitization, adenovirus type 5 (prototype strain) grown in KB cells was harvested after the cells were fully destroyed by the virus. To insure further release of virus, the preparation was treated by four cycles of freezing and thawing. This preparation contained $10^{6.5}$ to 10^6 TCID₅₀/ml of infectious virus measured after 7 days in KB tube cultures. Such preparations usually contained 16 units of CF antigen. The supernatant fluid was cleared of cellular debris by centrifugation at $800 \times g$ for 15 min. For animal immunization, adenovirus was grown in human fetal skin cells (HuFS), and harvested and treated as above. In addition, the virus was centrifuged for 4 hr at $78,000 \times g$ and resuspended in 10 or 5% of the original volume in Hanks' balanced salt solution. The concentrated viral antigen preparation used for animal immunization will be referred to as undilute, and dilutions of same will be expressed as \log_{10} .

Antisera. In the first experiment, rabbits weighing 2.5 to 3.5 kg were immunized with a single intravenous injection of 2 ml of virus into the marginal ear vein. Rabbits 1, 2, 3, and 4 received 20-fold concentrated tissue culture fluids. Rabbits 5 and 6 were injected with a 10^{-1} dilution, and rabbits 7 to 8 were given a 10^{-2} dilution of the concentrated preparation. Secondary responses were initiated in the same manner, except that a dilution of 10^{-1} of concentrated virus was used for all rabbits in this group regardless of primary

injection. A second experiment was set up with the use of 31 animals exposed to various levels of antigen. Rabbits 9 through 12 received the concentrated undiluted virus; 13 through 15 received 10^{-1} ; 16 through 27 received 10^{-2} , and 28 through 39, 10^{-3} dilution of concentrated preparation. Thirteen rabbits whose antibody titers were less than 1:10,240 at 66 days were selected for secondary challenge with adenovirus type 5. Four dilutions of antigen were used to elicit secondary responses: 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4} . Rabbits were bled at intervals depending on the experiment. All sera were inactivated at 56 C for 30 min and absorbed with sheep RBC to remove naturally occurring agglutinins.

Test procedure. Methods used were essentially those of Stavitsky (9) and can be summarized as follows. Sheep RBC were washed three times in saline and resuspended as 2.5% suspension. Equal volumes of cell suspension and tannic acid, 1:20,000 in phosphate-buffered saline (pH 7.2), were incubated for 10 min at 37 C. The cells were centrifuged, washed, and diluted to original volume, after which four additional volumes of buffered saline (pH 6.4) and one volume of antigen were added. In these experiments, it was found that 1:64 dilutions of viral antigen would give satisfactory titers when coated on sheep RBC. Higher concentrations would occasionally give a nonspecific agglutination. Therefore, 1:64 viral antigen was used throughout these experiments. After incubation of the cells for 10 min at room temperature, they were washed and resuspended in buffered saline (pH 7.2) containing 1:100 normal rabbit serum. Thimerosal (1:10,000) was added to saline stocks, which were stored at 4 C. The sera were heated to 56 C for 30 min and were absorbed with sheep RBC. In several experiments, an absorption of sera with KB cells was carried out to remove antibodies to the tissue antigen. This absorption was accomplished by adding a suspension of intact or lysed KB cells to the serum, followed by a centrifugation of cellular material. A second equally effective method was employed which allowed antisera to absorb on washed, intact KB cell sheets. Serum dilutions were made in twofold steps in tubes (13 by 100 mm). To each dilution in a volume of 0.5 ml was added 0.05 ml of 2.5% RBC suspension coated with antigen. Agglutination patterns were read after 3 hr and overnight incubation with 2+ considered as the end point.

In addition to the usual controls which accompanied each test, the specificity of the reaction was tested by means of the hemagglutination inhibition (blocking) test with the use of the specific adenovirus type 5 antigen as well as the background antigen contained in KB cells. RBC coated with adenovirus types 3 and 12 were used to test further the specificity of the adenovirus 5 antisera.

Comparative CF tests by use of a microtechnique in fourfold serum dilutions were run as follows. An amount of 2 units of guinea pig complement contained in a 0.05-ml volume was added to a mixture containing 0.025 ml of antigen and 0.025 ml of antiserum. After overnight incubation at 4 C, the amboceptor at a dilution of 1:1,000 was mixed with an equal volume of 2% sheep RBC, and 0.05 ml was added to each test

preparation. The preparation was incubated at 37 C for 1 hr and read for hemolysis, with 2+ considered as the end point.

NT in KB tissue cultures were done with certain representative sera as follows. A 1-ml amount of serum dilution was incubated with 1 ml of adenovirus type 5 containing 100 TCID₅₀ of virus. After 1 hr at 25 C, 0.2 ml of the mixture was inoculated into each of four KB tube cultures and read for cytopathic effects (CPE) during a 10-day period.

Determination of antibody species. Sucrose density gradient centrifugation was carried out to determine the type of antibody produced by rabbits during primary and secondary responses to adenovirus type 5. Two sucrose solutions were prepared at 37 and 10%. These solutions were mixed in proportion to give five additional intermediate concentrations differing by 4.5%. Gradients were prepared by carefully layering these dilutions in 0.6-ml increments, starting with the 37% dilution. The gradients were allowed to stand at 4 C for 2 hr until the interfaces between layers disappeared. The test serum was diluted with an equal volume of phosphate buffer (pH 7.2), and 0.4 ml of the mixture was carefully added to the top of the gradient. The gradients were centrifuged in a SW 39 rotor in a Spinco model L centrifuge for 16 hr at 100,000 × g. After centrifugation, 11 fractions were collected from the top by removing approximately 0.4-ml samples with a blunt needle from the center of the tube just below the meniscus. The fractions were numbered 1 through 11, starting at the top of the tube, and were diluted in twofold steps with 1:100 normal rabbit serum in buffer (pH 7.2) tested by use of tanned cells coated with adenovirus type 5 as described above. It should be noted that fractions were not dialyzed, since it was observed that sucrose did not interfere with hemagglutination.

Sera were screened for the presence of 2-mercaptoethanol (ME)-sensitive antibody. They were diluted 1:10 with buffer and incubated with an equal volume of 0.2 M ME for 1 hr at 37 C, after which they were placed at 4 C overnight. They were then assayed directly by hemagglutination. Selected sera were treated by dialysis against 0.1 M ME according to the method of Deutch and Morton (2), followed by sucrose gradient centrifugation. After treatment, the fractions were tested by hemagglutination as above. The former procedures will be referred to as the direct method and the latter as the indirect method.

RESULTS

With the PHA technique, antibody titers of greater than 1:10 were detectable in rabbit sera as early as 3 days after the primary injection in rabbits 3, 5, and 6 (Table 1). In some instances, serum titers of 1:10,240 and 1:20,480 were obtained from a single primary injection. Rabbits 7 and 8, which received the smallest dose of adenovirus antigen, required the most time for the appearance of antibody (9 days), and their titers were considerably lower than the titers of animals immunized with a 100 times greater

TABLE 1. Antibody response* of rabbits to a single injection† of adenovirus type 5 as detected by passive hemagglutination (PHA), complement fixation (CF), and neutralization (NT)

| Days after immunization | Rabbit No. | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------------|------------|----|-----|-------|-----|-----|--------|-----|-----|--------|-----|-----|-------|-----|-----|-------|-----|-----|-------|-----|-----|-----|--|--|
| | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | | 7 | | | 8 | | | | | |
| | PHA | CF | NT | PHA | CF | NT | PHA | CF | NT | PHA | CF | NT | PHA | CF | NT | PHA | CF | NT | PHA | CF | NT | | | |
| 0 | <10 | <4 | <10 | <10 | <4 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | | |
| 2 | <10 | <4 | <10 | <10 | <4 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | | |
| 3 | 1,280 | <4 | 20 | 640 | <4 | 20 | 160 | 16 | <10 | 40 | 4 | <10 | 320 | 4 | <10 | 40 | 4 | <10 | 40 | 4 | <10 | <10 | | |
| 4 | 1,280 | <4 | 20 | 640 | <4 | 20 | 1,280 | 16 | <10 | 40 | 4 | <10 | 320 | 4 | <10 | 40 | 4 | <10 | 40 | 4 | <10 | <10 | | |
| 5 | 10,240 | 32 | 20 | 2,560 | 128 | 20 | 5,120 | 64 | 10 | 640 | 4 | 10 | 1,280 | 64 | <10 | 1,280 | 64 | <10 | 1,280 | 64 | <10 | <10 | | |
| 6 | 10,240 | 32 | 20 | 2,560 | 128 | 20 | 5,120 | 64 | 10 | 640 | 4 | 10 | 1,280 | 64 | <10 | 1,280 | 64 | <10 | 1,280 | 64 | <10 | <10 | | |
| 9 | 10,240 | 64 | 80 | 5,120 | 256 | 80 | 10,240 | 32 | 20 | 10,240 | 32 | 40 | 640 | 64 | <10 | 2,560 | <4 | 20 | 2,560 | <4 | 20 | <10 | | |
| 12 | 10,240 | 64 | 80 | 5,120 | 256 | 80 | 10,240 | 32 | 20 | 10,240 | 32 | 40 | 640 | 64 | <10 | 2,560 | <4 | 20 | 2,560 | <4 | 20 | <10 | | |
| 23 | 10,240 | 64 | 80 | 5,120 | 256 | 80 | 10,240 | 32 | 20 | 10,240 | 32 | 40 | 640 | 64 | <10 | 2,560 | <4 | 20 | 2,560 | <4 | 20 | <10 | | |
| 25 | 10,240 | 64 | 80 | 5,120 | 256 | 80 | 10,240 | 32 | 20 | 10,240 | 32 | 40 | 640 | 64 | <10 | 2,560 | <4 | 20 | 2,560 | <4 | 20 | <10 | | |

* Expressed as reciprocal of the serum dilution.
 † Rabbits 1 to 4 received a 10⁶ dilution of antigen; rabbit 5, 10⁻¹; rabbits 7 and 8, 10⁻².

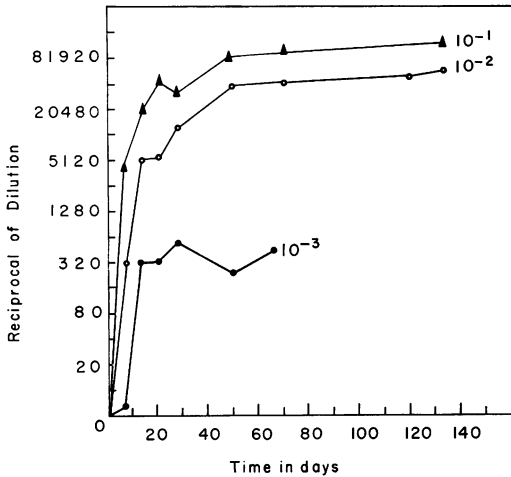


FIG. 1. Primary antibody response of rabbits to dilutions of adenovirus type 5.

amount of antigen. In practically all instances, the HA antibodies were demonstrable earlier than the CF and NT antibodies, and they were present in considerably higher titers.

Six rabbits were restimulated between days 23 and 130 after primary immunization. At the time of the secondary inoculation, these animals had titers ranging from 1:320 to 1:20,480. The second inoculation caused a further antibody increase, first observed on day 3 after restimulation. The highest secondary antibody was obtained in the animal with the lowest initial titer (1:320), which in the course of 3 days became elevated to 1:20,480. Two animals were given a tertiary stimulation, one on day 58 and the other on day 94 after primary stimulation. This injection caused a further increase in antibody titers beginning on day 3 and reaching the maximum on day 7; the maxima corresponded to titers to 1:163,840 in one rabbit and 1:327,680 in the other.

To determine more precisely the time and dose effects on the synthesis of antiviral hemagglutinating antibodies, a large-scale experiment was designed utilizing 31 rabbits and varying concentrations of primary antigenic material. At 71 days after primary stimulation, some of these rabbits were given a secondary injection consisting of one of three different concentrations. The results obtained with the primary immunization are presented in Fig. 1. These data were previously submitted to probit analysis by plotting probits versus logarithms of the arithmetic mean-titer (arithmetic mean on log scale, Fig. 2). The distribution of points is in a straight line, indicating that the use of arithmetical means in the

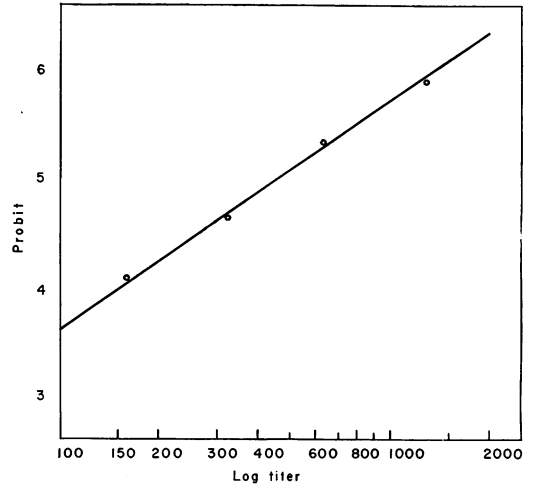


FIG. 2. Probit analysis of the distribution of the antibody titer of rabbits, 7 days after primary injection of 10^{-2} antigen.

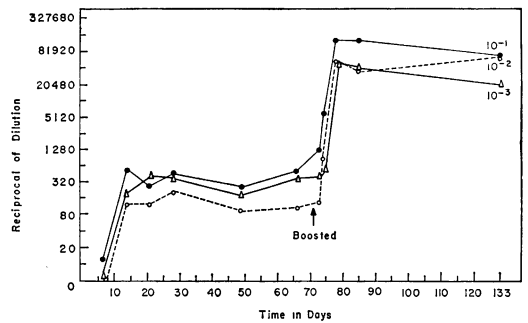


FIG. 3. Secondary antibody responses of rabbits boosted on day 71 with various dilutions of antigen.

presentation of data such as shown in Fig. 1 and 3 is justified. The results in Fig. 1 clearly show that the development of hemagglutinating antibodies was dependent on the dose of antigen.

Nine rabbits which were previously inoculated with the lowest concentration of antigen, and at 66 days had serum titers below 1:10,240, were divided into three groups for secondary inoculation. On day 71, each of the groups was injected with 10-fold diminishing amounts of adenovirus from the same preparation employed in the primary immunization. The results presented in Fig. 3 represent an average of three animals, and show that the animals responded vigorously to the restimulation. Significant antibody rises were already evident in 3 days, and maximal titers were obtained at 7 days. The amount of antibody at this time was 100 to 500 times greater than at the time of secondary inoculation. At times, titers of

TABLE 2. Effect of antigen concentration* on antibody titer† of rabbits boosted on day 71 as measured by passive hemagglutination

| Days after secondary immunization | Rabbit no. | | | | | | | | |
|-----------------------------------|------------|-------|-------|-----------|-----------|-------|---------|--------|---------|
| | 37 | 39 | 31 | 34 | 38 | 29 | 33 | 35 | 36 |
| 7 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 |
| 14 | 160 | <10 | <10 | 160 | 640 | <10 | 640 | 40 | 640 |
| 21 | 320 | <10 | <10 | 160 | 640 | <10 | 640 | 640 | 640 |
| 28 | 320 | <10 | <10 | 160 | 640 | <10 | 640 | 640 | 640 |
| 49 | 320 | <10 | <10 | 320 | 2,560 | <10 | 1,280 | 40 | 2,560 |
| 66 | 1,280 | <10 | <10 | 640 | 5,120 | <10 | 2,560 | 160 | 2,560 |
| 73 | 1,280 | <10 | <10 | 1,280 | 5,120 | <10 | 2,560 | 320 | 5,120 |
| 74 | 40,960 | 160 | 40 | 2,560 | 10,240 | 10 | 2,560 | 160 | 5,120 |
| 78 | 327,680 | 5,120 | 2,560 | 327,680 | 1,310,720 | 5,120 | 327,680 | 40,960 | 327,680 |
| 85 | 327,680 | 5,120 | 2,560 | 163,840 | 1,310,720 | 2,560 | 327,680 | 20,480 | 327,680 |
| 133 | 40,960 | | 2,560 | 1,310,720 | 655,360 | 5,120 | 327,680 | | 81,920 |

* Rabbits 37 and 39 received a 10⁻¹ dilution of antigen; rabbits 31, 34, and 38, 10⁻²; rabbits 29, 33, 35, and 36, 10⁻³.

† Expressed as a reciprocal of dilution.

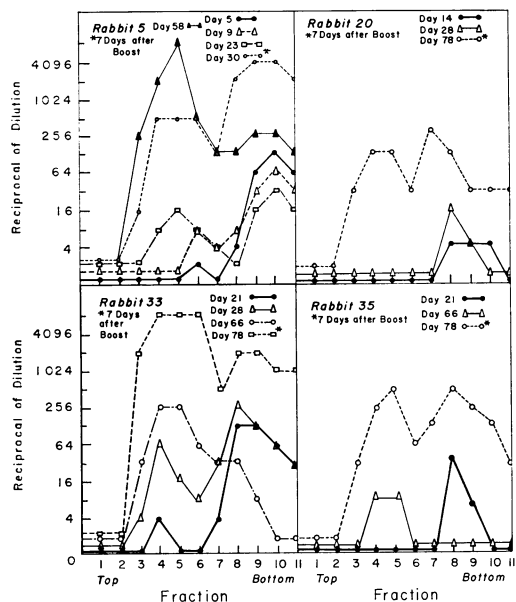


FIG. 4. Sucrose gradient ultracentrifugation of rabbit sera before and after secondary stimulation.

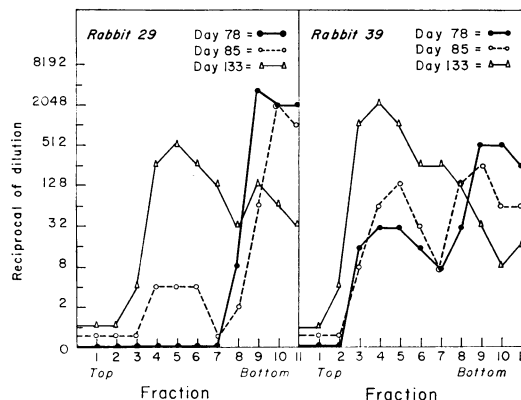


FIG. 5. Sucrose gradient ultracentrifugation of rabbit sera showing the effect of virus concentration on secondary responses of primed animals.

1:1,310,720 were obtained. In contrast to the primary response, the secondary response to the small amount of antigen contained in the 10⁻³ dose appeared to be very similar to the response obtained with the higher concentrations of antigen. Table 2 shows the individual titers. In addition to revealing the high magnitude of the secondary response, it shows the results of the sera of three rabbits which initially failed to produce measurable antibody (no. 39, 31, and 29), but

which responded adequately to secondary stimulation, reaching titers of 1:2,560 to 1:5,120. Antibody titers persisted for over 6 months (not shown) with the unboosted rabbits without significant decrease. This persistence of antibody was also noted with the boosted animals.

Studies on the nature of antibody. The PHA antibodies produced after primary and secondary immunization were subjected to physicochemical analysis. The sedimentation of antibody molecules in a sucrose gradient was determined for six rabbits. The results obtained with multiple sera from these rabbits are given in Fig. 4 and 5. Rabbit 5 was injected with 10⁻¹ dilution of antigen. The early serum samples from this animal appeared to contain primarily heavy (19S) anti-

bodies, followed by subsequent appearance of the lighter (7S) component. A 30-day sample taken 7 days after secondary stimulation with an equivalent amount of antigen showed a significant increase in antibody level in both the heavy and light fractions. A sample taken at 58 days indicated a predominance of slow sedimenting antibody. Rabbit 20, which was exposed to a 10^{-2} dilution of antigen, developed only 19S antibody on primary stimulation. Secondary injection of 10^{-2} antigen resulted in an increase in both the heavy and light antibodies, similar to that which occurred with animal 5. Rabbits 33 and 35 received a 10^{-3} dilution of antigen in both the primary and secondary injections. A pattern of antibody synthesis similar to that which occurred in animal 5 was noted, that is a sequential production of 19S followed by 7S, both of which increased after secondary stimulation. Rabbits 29 and 39 (Fig. 5) were initially immunized with antigen diluted 10^{-3} . The former was restimulated with an antigen dilution of 10^{-3} and the latter with 10^{-1} . It will be recalled that neither rabbit showed any demonstrable antibodies prior to the second inoculation. In rabbit 29, the peak titer

was obtained on day 7 after secondary (78 days after primary) inoculation (1:5,120). These antibodies were all concentrated in the heavy fraction. There was evidence of light antibody 7 days later, but the heavy antibodies predominated. At 133 days, there was a definite shift toward light antibodies, the amount of which exceeded that of the heavy antibodies. Rabbit 39, which also failed to show antibodies prior to secondary injection, but which was injected secondarily with a larger amount of antigen on day 71 (10^{-1}), already showed a significant amount of light antibodies at day 7 after restimulation and by day 133 showed predominantly, if not exclusively, light antibodies. The response of rabbit 31 (not shown), which was restimulated with 10^{-2} dilution, was qualitatively intermediate between rabbit 29 and 39.

Specificity of the reactions is demonstrated in Table 3, which features the results obtained with sera from three rabbits collected on days 71 and 101. The antiviral hemagglutinating titers were quite high (1:163,840). It can be readily seen that when free adenovirus type 5 was added to these sera it significantly lowered the titer for the adenovirus-coated RBC. KB antigen, when added to these sera prior to reaction with adenovirus-coated RBC, caused little change in titer. This table shows also that these sera had relatively low titers against the KB antigen which could be blocked by either KB or KB-grown adenovirus 5 antigens.

The clear separation of antiviral antibodies from the anti-KB antibodies is also illustrated in Table 4, in which absorption with KB cells did not significantly influence the adenovirus 5 titers but caused appreciable reduction in anti-KB activity.

Treatment with ME. Several serum samples collected at different time intervals from rabbit 20 were treated with ME by the direct procedure. After this treatment the sera were tested in the

TABLE 3. Reactivity of sera* after the addition of excess antigens

| Test antigen | Free antigen | Rabbit no. | | |
|--------------|--------------|------------|---------|---------|
| | | 2 | 3 | 5 |
| | | Day 101 | Day 71 | Day 71 |
| Adeno 5 † | | 163,840 | 163,840 | 163,840 |
| | Adeno 5 | <640 | <640 | 2,560 |
| KB | KB | 163,840 | 327,680 | 81,920 |
| | Adeno 5 | 2,560 | 5,120 | 20,480 |
| | Adeno 5 | <640 | <640 | <640 |
| | KB | <640 | <640 | <640 |

* Expressed as reciprocal of dilution.

† Adenovirus type 5.

TABLE 4. Reactivity of sera* after absorption with intact KB cells

| Test antigen | Rabbit no. | | | | | | | |
|--------------|------------|----------|------------|----------|------------|----------|------------|----------|
| | 3 | | 5 | | 8 | | 8 | |
| | Day 6 | | Day 30 | | Day 78 | | Day 85 | |
| | Unabsorbed | Absorbed | Unabsorbed | Absorbed | Unabsorbed | Absorbed | Unabsorbed | Absorbed |
| Adeno 5 † | 10,240 | 5,120 | 2,560 | 10,240 | 81,920 | 81,920 | 40,960 | 20,480 |
| KB | 160 | <40 | 160 | <40 | 2,560 | <80 | 640 | <80 |

* Expressed as reciprocal of dilution.

† Adenovirus type 5.

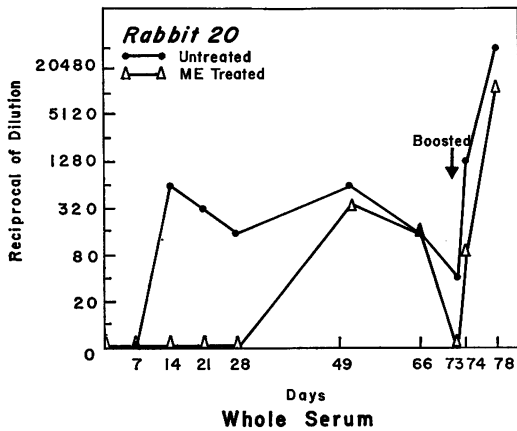


FIG. 6. Sensitivity of rabbit adenovirus antibody to 2-mercaptoethanol (ME).

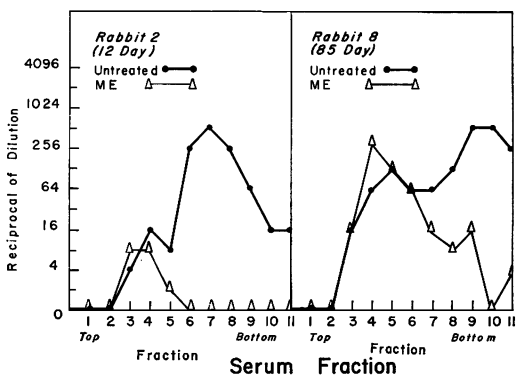


FIG. 7. Sensitivity of rabbit adenovirus antibody to 2-mercaptoethanol (ME) followed by sucrose gradient ultracentrifugation.

PHA. The results in Fig. 6 showed that prior to day 49 the hemagglutinins were entirely sensitive to ME treatment, but at this time and thereafter most of the antibody activity was associated with the ME-resistant component. Two sera, one taken 12 days after primary stimulation of rabbit 2 and the other taken 14 days after secondary stimulation (85 days after primary stimulation) of rabbit 8, were exposed to ME by the indirect procedure. These treated sera, along with corresponding untreated controls, were subsequently placed on a sucrose gradient and centrifuged at $100,000 \times g$. The results are included in Fig. 7. The 12-day-old serum lost most of its activity after treatment, and whatever activity persisted was located in the light fraction. Conversely, the serum taken late in the immunization contained a large amount of ME-resistant antibody.

DISCUSSION

It has been recognized for some time that the PHA test is a highly sensitive serological procedure for measuring antibodies to protein antigens (9). The present report demonstrates that this procedure is also highly sensitive in the detection of antibodies engendered by viral antigens. This procedure was found to be capable of detecting antibodies earlier than the conventional CF and NT tests. Moreover, the antibody titers obtained in the PHA were 10 to 100 times greater than the corresponding antibody titers obtained in the standard serological procedures. At the present time, it is not possible to assign a form of specificity to the PHA test as to whether it is type- or group-specific. In a few preliminary experiments, antiserum to adenovirus type 5 did not agglutinate RBC coated with adenovirus types 3 and 12, nor did adenovirus type 3 block the agglutination of cells coated with adenovirus type 5 in the presence of adenovirus type 5 serum. These findings are, however, not conclusive, and the work of others with human sera has pointed to the occurrence of cross-reactions (3, 7). Prier and LeBeau (6) observed that the PHA test provided a higher degree of specificity than did the CF or conglutination tests.

In line with the findings of Uhr and Finkelstein (12) and Svehag and Mandel (10), who demonstrated the formation of 19S and 7S antibodies to bacteriophage and poliovirus, the sera produced in the present experiments in response to immunization with adenovirus type 5 contained both types of antibodies. These investigators showed that secondary responses to antigens are primarily 7S antibodies. Svehag and Mandel (11) found that only when secondary stimulation with antigen occurred within a few days after the cessation of 19S antibody synthesis were anamnestic 19S responses observed. In the present studies, it was shown that the PHA test measures both heavy and light antibodies. The former predominate during the early phase of immunization. By the use of this test it was also possible to demonstrate renewed synthesis of heavy antibody in addition to light antibody after the secondary immunization. A rabbit which failed to respond to the primary injections of a low dose of antigen, but which responded to a secondary injection of a similar low dose, produced antibodies in secondary responses which were predominantly of the 19S variety. With time and in the absence of further stimulation, the heavy antibodies were replaced to a large extent by light antibodies. A significantly earlier production of 7S antibodies was manifested in a rabbit restimulated with a higher dose of antigen after primary injection of a

low dose of antigen which caused no demonstrable antibodies.

The primary immunization was much more dose-dependent than was the secondary immunization. This was evident both in the amount and type of antibody synthesized. In general, delayed formation of 7S antibody was observed when minimal amounts of antigen were used.

It is interesting to note that antibodies to adenovirus type 5 persisted in rabbits for many months after either primary or secondary stimulation. This was also shown with the polio and bacteriophage systems previously described and is associated with the slower sedimenting of antibody fraction. The reason for this prolonged maintenance of high levels of antibody is not known, but it is conceivable that it may be associated with survival of this virus in the rabbit. It was previously reported by Pereira and Kelly (5) that this virus sets up a latent infection in rabbit spleens for a period of at least 2 months.

ACKNOWLEDGMENTS

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