# A NEW TECHNIQUE FOR EVALUATING ANTIGENIC RELATEDNESS AMONG VIRUSES.

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# A NEW TECHNIQUE FOR EVALUATING ANTIGENIC RELATEDNESS AMONG VIRUSES

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13 ABSTRACT

We previously reported the ability of the macrophage migration inhibition (MMI) test for differentiating both bacterial viruses and two cross-reacting strains of the avian coronavirus, infectious bronchitis virus. During the past year, we have demonstrated that Adenovirus types 1, 4, 5 and 7 are all clearly separable by MMI test procedures. It was also determined that the optimal sensitizing dose for Adenovirus type 1 was 100 ugm per pig and that doses above or below this level gave poorer responses. In addition, two nuclear polyhedrosis viruses (NPV) which infect the alfalfa looper and cabbage looper (two important insect pests) respectively were clearly differentiated by MMI test procedures. The polyhedral sizes, shapes and virion occlusion patterns of the two species of NPV were indistinguishable.

Attempts to passively transfer the delayed response to normal peritoneal exudate cells by using RNA extracts of tissues from sensitized animals have thus far been unsuccessful.

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ABSTRACT

The macrophage migration inhibition test (MMI) was found to be an effective means of differentiating two nuclear polyhedrosis viruses (NPV) which infect the following insects, the cabbage looper (Trichoplusia ni) and the alfalfa looper (Autographa californica). Peritoneal exudate cells from guinea pigs sensitized to T. ni virions demonstrated significant MMI in the presence of T. ni virions but not with the A. californica antigen. Similarly, when A. californica virions were employed as sensitizing antigen, only the homologous antigen gave a significant MMI response. These two insect viruses are not separable by any other technique.

Adenovirus types 1, 4, 5, and 7 were also clearly separated by MMI test procedures. Inhibition of macrophage migration was always greatest when the test antigen employed was homologous to the sensitizing antigen. Studies on the effect of the concentration of sensitizing antigen employed showed that the optimal dose for Adenovirus type 1 was 100 µgm per pig. Concentrations above this level induced a poorer MMI response probably due to the production of humoral antibody whose presence is known to interfere with the induction of delayed hypersensitivity.

Attempts to passively transfer the delayed response to normal peritoneal exudate cells by using RNA extracts of spleen and lymph node tissues of pigs giving good MMI response (to either the Adenovirus types or the two NPV's) have thus far been unsuccessful.

## INTRODUCTION

The first year of the contract work involved establishing the optimal conditions needed for performing macrophage migration inhibition (MMI) tests in our laboratory. The initial studies were performed with the P 22 and P 221 phage of Salmonella typhimurium. Highly purified preparations of P 22 and P 221 phage were used to sensitize Hartley strain guinea pigs. Following sensitization, guinea pig peritoneal exudate cells were placed in capillary tubes and the degree of MMI in the presence of the two antigens was measured. In all tests, the migration of macrophages was inhibited to a greater degree by the sensitizing antigen than by the heterologous antigens. A dose response curve for migration inhibition caused by increasing concentrations of P 22 protein used for sensitization was established. The P 22 and P 221 bacteriophages were clearly distinguished with good statistical significance as measured by Student's paired t test. P 221 proved to be a more effective antigen in eliciting cellular hypersensitivity reactions than P 22.

The MMI test was also able to distinguish between two serologically cross-reacting strains of infectious bronchitis virus (IBV), an avian coronavirus. A dual system for propagating IBV was used for the sensitizing and test antigen preparations. Following guinea pig immunization with chicken tracheal explant grown IBV-1, the MMI test was able to differentiate IBV-1 from either IBV strains 8 or 10 using embryonated egg propagated test antigen. No significant MMI was observed with any of the control peritoneal exudate cells studied. Neither nonsensitized cells in the presence of the test antigens nor sensitized cells in normal media showed any significant reduction in their ability to migrate.

In the report to follow, we describe results obtained from MMI studies with four human Adenoviruses and with two insect viruses which may prove to be of importance in the control of two insect pests. The results of experiments designed to determine the effect of the amount of virus employed for the sensitization of guinea pigs are described as are the results of attempts to transfer the delayed response to normal guinea pig peritoneal exudate cells by using RNA extracts of tissues from animals giving good MMI responses to either the Adenovirus serotypes or the two insect viruses.

### (A) Studies with Adenoviruses

The specificity and sensitivity of the macrophage migration inhibition test in differentiating between virus serotypes has not been widely studied. The virus species selected for this study were Adenovirus types 1, 4, 5, and 7. The capsid of human adenoviruses has been shown to consist of three major structural components, which correspond to the viral antigens: 1) hexons which contain the group specific CF antigen, 2) fibers which represent a type-specific antigen, and 3) pentons which are associated with viral hemagglutination.

Four subgroups have been distinguished among the human adenoviruses based on the ability to agglutinate rhesus monkey and rat erythrocytes. Adenovirus types 1, 4, and 5 are members of one subgroup, and type 7 is in another group.

Parks, et al., (1967) have shown that the subdivisions based on HA characteristics appear to parallel immunological groupings. Inoculation with one serotype induces antibody development to other members of the subgroup as well as to the type which was injected with the degree of cross-reactivity varying with the serological technique employed.

The purpose of this study is to determine whether the macrophage migration inhibition test can be used to differentiate between serologically-related adenovirus types and to study several parameters which might influence the sensitivity and specificity of the procedure.

## MATERIALS AND METHODS

### A. Preparation of Adenovirus Sensitizing Antigens

1. Source. The Adenovirus types 1, 4, 5, and 7 virus stocks were kindly supplied by Elizabeth J. Sullivan, Virology Division, Naval Medical Research Unit No. 4, Great Lakes, Illinois. Adenovirus types 1 and 5 were prototype strains and had a cell culture passage history of three passages in HEp-2 cells and one passage in HeLa cells. Adenovirus type 4 was the vaccine strain with a passage history of HEK 3 HeLa 1 while the Adenovirus type 7 was a Great Lakes isolate which had one passage in HEp-2 and one passage in HeLa cells.

2. Propagation. The four strains of adenovirus were propagated in KB cell cultures which were infected with virus inocula as stationary monolayers in 32-ounce bottles or as monolayer cultures in half-gallon roller bottles.

The growth medium was decanted off when confluent KB monolayer cultures were established and monolayers were infected with adenovirus pools which had been titrated in HeLa cells. Infected cultures were maintained with EBME containing 2% fetal calf serum and were incubated at 37 C until approximately 80% of the cells showed cytopathic effects. The virus purification scheme employed is described in Fig. 1. The figure shows only one cycling of the viruses through cesium chloride gradients (steps 5-7). Actually, each virus preparation was cycled through three CsCl gradients to insure that highly purified preparations were obtained. With several of the preparations, a second band was evident at a density of 1.41 following the first cycle through CsCl and probably represented an Adeno-associated virus (AAV) contaminant. No such bands were seen in any of the subsequent CsCl runs.

### B. Preparation of Adenovirus Test Antigens

Adenovirus MMI test antigens were prepared from the virus stocks described previously. The cell suspensions were freeze-thawed three cycles and centrifuged at 2,000 x g for 10 min to remove cellular debris. The supernatant fluids were assayed for infectivity by the tube titration method using KB cell monolayer cultures. The virus concentration of each adenovirus pool was diluted to an approximate titer of $10^{5.4}$ to $10^{5.6}$ $TCID_{50}$ per ml. The test antigen used for MMI procedures consisted of one volume of the virus suspension plus one volume of a 2X concentration of HBME containing 30% guinea pig serum ($HBME_{70}GPS_{30}$).

C. Macrophage Migration Inhibition Test (MMI)

1. Sensitization of guinea pigs. Hartley strain albino guinea pigs, weighing 250 to 350 g were obtained from Camm Research Institute, Wayne, New Jersey. The sensitizing antigens consisted of the purified adenovirus preparations. The protein content of each preparation was determined by the method of Lowry (1951) and adjusted to the desired concentrations with sterile PBS. The antigens were emulsified in an equal volume of complete Freund's adjuvant and guinea pigs were sensitized by distributing the antigen dose into each of three sites, the two hind foot pads and a lateral intradermal site, using 0.1 ml of sensitizing antigen per site. The protein content of each adenovirus antigen varied from 25 to 250 mg total dose depending on virus type and experimental procedure employed.

2. Preparation of peritoneal exudate cells (PEC). Peritoneal exudate cells were harvested 21 to 30 days following injection of the guinea pigs with adenovirus sensitizing antigen. Seven days prior to harvesting the PEC, sterile mineral oil (Squibb) was injected intraperitoneally using a volume of 20 ml for each animal. The peritoneal exudate cells were collected by syringe and allowed to separate into a cell-containing fraction and an oil fraction in a sterile separatory funnel. The cells were collected in sterile centrifuge tubes and were sedimented by centrifugation at 700 x g for 3 min at 4 C. The cells were washed three times in EBSS containing 1% normal guinea pig serum at 700 x g for 5 min at 4 C. After the final wash, the cells were resuspended in 2-3 volumes of Eagle's Basal Medium (EBME) with EBSS and 15% pooled normal guinea pig serum.

3. Preparation of migration capillary tubes. The cells were then used to fill 0.7 mm x 75 mm capillary tubes. One end of each tube was sealed with a plug of Critoseal (Scientific Products) after which the cells were sedimented onto the Critoseal plug by centrifugation at 200 x g for 5 min. The tubes were broken at the cell-medium interface by means of a sterile forceps and the tubes were placed into cell culture dishes (four per dish) and 3.0 ml of EBME with 15% guinea pig serum and either an adenovirus pool prepared in KB cells or a KB cell sonicate control was added to each plate. The tissue culture dishes containing the test antigens were incubated at 37 C in a humidified 5% $CO_2$ atmosphere for 18 hr.

4. Evaluation of migration inhibition. Determination of the degree of inhibition was made by measuring the area of the "fan" of macrophages which migrate in radial fashion from the capillary tube onto the tissue culture dish surface. The migration area was traced on paper using a viewing screen attached to an American Optical Microstar microscope and the area of migration determined by planimetry. The MMI was calculated by using the average of four planimeter readings obtained from macrophage migrations obtained in the presence of adenovirus test antigens and from readings obtained from macrophage migrations observed in the presence of control KB sonicates. The percentage migration or migration index (MI) was expressed as the control migration being equal to 100%. The per cent macrophage migration inhibition (% MMI) was found by using the formula:

$$\% \text{ MMI } 1 - \frac{\text{Average area of migration with antigen}}{\text{Average area of migration without antigen}} \times 100$$

## RESULTS

The first series of experiments were conducted to determine if adenovirus protein, at a concentration sufficient to induce the delayed hypersensitivity response, could be produced by using the selected purification procedure, and whether a specificity existed when PEC sensitized with one adenovirus type antigen were incubated with the homologous test antigen.

Adenovirus type 1 was the initial serotype which was studied. Table 1 presents macrophage migration inhibition data obtained when guinea pigs were sensitized with 70 or 120 mg of Adenovirus type 1 protein in complete Freund's adjuvant. The % MMI values were calculated from an average of four migration inhibition values for each antigen and for the control migration test. The % MMI's observed with Adenovirus type 1 test antigen were, in all six preparations, greater than those observed when Adenovirus types 4, 5, and 7 test antigens were used.

The results obtained using cells from pigs sensitized with Adenovirus type 4 antigen and tested with Adenovirus types 1, 4, 5, and 7 antigens are presented in Table 2. In five of six preparations tested, the % MMI obtained in the homologous test system using Adenovirus type 4 test antigen was greater than with the other antigens.

Table 3 presents % MMI data obtained with cells from pigs sensitized with Adenovirus type 5 antigen. Again, a greater % MMI was observed in the homologous test system than with the heterologous viral antigens.

The results for the Adenovirus type 7 macrophage migration system are presented in Table 4. Again, a larger % MMI was observed when Adenovirus type 7 sensitized PEC were exposed to the homologous test antigen than that obtained with the heterologous Adenoviruses 1, 4, and 5.

In an attempt to decrease the guinea pig-to-guinea pig variations which were observed using Hartley strain guinea pigs, a series of experiments using NIH strain 13 guinea pigs was conducted. Strain 13 guinea pigs are derived from an inbred colony and do not exhibit an immunogenetic disparity between members of the colony. The observed histocompatibility properties of this strain allowed the use of pooled PEC preparations for MMI studies. Table 5 presents the % MMI results which were obtained when Adenovirus type 1 sensitized PEC were treated with Adenovirus types 1, 4, 5, and 7 antigens, as individual animal preparations and as a 3-animal PEC pool. The % MMI did not appear to be significant in the homologous antigen system and the % MMI's observed with Adenovirus types 4, 5, and 7 were actually greater than that observed using type 1 antigen in the 13/1 PEC preparation. No advantage of using pooled cells was detected.

Table 6 presents an analysis of variance calculated for the Adenovirus types 1, 4, 5, and 7 groups and for the Strain 13 experiments. The F-Test is a test of differences between population means and was used to determine the probability of significant differences between % MMI produced by the four test antigens. As a matter of practical convenience, probability levels of 5% were used in deciding whether to reject a hypothesis of no difference between the population means. A probability less than 5% indicated a significant difference

existed between the mean values of % MMI produced by each adenovirus test antigen and suggested that a specific inhibition of migration occurs when PEC are incubated in the presence of homologous test antigen.

A series of experiments was conducted to study the effect varying concentrations of sensitizing antigen had on MMI test results. The % MMI results obtained using Adenovirus type 1 sensitizing doses of 25, 50, and 100 µg are shown in Table 7. Significant MMI was noted only in PEC sensitized with 100 µg of antigen. A lack of specificity was observed when 25 and 50 µg doses were used as the % MMI values were similar for both the homologous and heterologous systems and, in some cases, values for the heterologous system were larger.

Table 8 presents data obtained from guinea pigs sensitized with 150, 200, and 250 µg doses of Adenovirus type 1 antigen. The decrease in % MMI specificity which was observed by administering low-concentration sensitizing doses was again observed when sensitizing doses greater than 150 µg of Adenovirus type 1 protein were used. Paired sera obtained at the time of sensitization and at the time of harvesting cells have been collected and will be tested for the presence of humoral antibodies. The presence of humoral antibody has been reported to interfere with the induction of the delayed hypersensitivity state. It is suspected that antibody synthesis may have been induced by the high levels of adenovirus antigen and that the presence of antibody may be responsible for diminishing the level of migration inhibition.

Table 9 presents the analysis of variance calculated from the dose-response data. A significant difference between the mean values of % MMI produced by each test antigen was obtained by using immunizing dose concentrations of 100 and 150 µg.

(B) Work with Insect Viruses

One of my graduate students, Mr. Charles Benson, has been studying insect viruses in the laboratory of Dr. Charles Reichelderfer in the Entomology Department here at the University. When it was determined that two of the viruses he was studying were indistinguishable with the methodology available, we decided to employ MMI test procedures to ascertain if, indeed, the two agents were identical or different antigenically. The results of the study are described below.

## MATERIALS AND METHODS

Virus stocks. The cabbage looper, T. ni MEV virus employed in this investigation was obtained from Dr. A. M. Heimpel, U.S.D.A. Insect Pathology Pioneering Research Laboratory, Beltsville, Maryland. The MEV virus of the alfalfa looper, A. californica was supplied by Dr. P. V. Vail, U.S.D.A., A.R.S. Western Cotton Research Laboratory, Phoenix, Arizona. These viruses were propagated in the fall armyworm, Spodoptera frugiperda, cleaned by the differential centrifugation method described by Lewis (1960), and standardized in terms of polyhedral inclusion bodies (PIBs)/ml of aqueous suspension.

Electron microscopy. All electron micrographs employed in the comparison of T. ni and A. californica NPV were prepared by Dr. Jean R. Adams, U.S.D.A.

Insect Pathology Pioneering Research Laboratory. The two insect viruses were compared as to PIB size and shape, pattern of occlusion of virions within polyhedra, and mean number of virions per developmental membrane.

Bioassay procedure. The two species of NPV were compared by dose-mortality bioassay with neonate cabbage looper and fall armyworm larvae (Thomas et al., 1972).

Dose-mortality data was subjected to probit analysis utilizing the maximum liklihood procedure of Finney (1952) to obtain the weighted linear regression of probits on log dosage. The observed responses were adjusted by Abbott's formula (1925). Application of Finney's procedure yielded an analysis of linear regression, LD values from 10 to 90, and 95% fiducial limits about each LD value.

Virus purification. Suspensions of virus that had been cleaned by differential centrifugation were further purified by sucrose density gradient centrifugation followed by isopycnic density gradient centrifugation in cesium chloride.

The sucrose gradient employed was linear from 40% to 70% sucrose, w/w, in 0.01M Tris-HCl buffer, pH 7.2. The virus was layered on the gradient in 0.5 ml aliquots containing $3.6 \times 10^8$ A. californica or T. ni PIBs and centrifuged at 32,400 x g for 60 min at 4 C in a Beckman Model L-2 preparative ultracentrifuge. Resultant PIB bands were collected and dialyzed overnight against distilled water at room temperature.

The refractive index of the resultant dialysate was raised to 1.3623 with CsCl utilizing a Bausch and Lomb Abbe-3L refractometer at 24 C. The suspension was then centrifuged at 100,000 x g for 18 hr at 4 C. Again, PIB bands were collected and dialyzed overnight. The volume of dialysate was adjusted to 20 ml with sterile distilled water. Purified PIB samples were standardized in terms of PIBs/ml.

PIB dissolution. Dissolution of polyhedral protein was accomplished by centrifuging 1 ml of a purified PIB sample at 12,350 x g for 10 min at 4 C. Pelleted PIBs were resuspended in 0.4 ml 0.05M NaCl - 0.6 ml 0.05M $Na_2CO_3$, pH 11.2, and agitated at frequent intervals for 60 min at room temperature. The suspension was diluted 1:9 with distilled water and 0.1 ml 1N HCl was added to lower the pH to 8.6. Undissolved polyhedra were removed from solution by centrifugation at 12,350 x g for 10 min at 4 C. Virions were sedimented from the resulting supernatant by centrifugation at 4 C for 75 min at 100,000 x g and resuspended in 0.001M NaCl. To remove any remaining bacteria, partially dissolved polyhedra or other contaminants, both polyhedral protein solutions and virion suspensions were passed through Millipore Swinnex Filter Units, 0.45 u pore size (Millipore Filter Corp.).

Protein determination. The total protein content of the polyhedral protein and virion preparations was determined against crystalline bovine serum albumin standards (Lowry et al., 1951).

Sensitization. Prior to inoculation, immunizing antigens were emulsified in an equal volume of complete Freund's adjuvant (CFA). Albino Hartley strain guinea pigs (Camm Research Institute) weighing 300 to 600 g were sensitized by injection of 0.2 ml of emulsified antigen into each of the hind footpads and

0.1 ml intradermally. A total of 80 µg protein per guinea pig was used uniformly as the inoculating dose. Control guinea pigs were injected with a 1:1 emulsion of sterile distilled water and CFA.

The recovery and preparation of cells for MMI testing were essentially the same as that described previously for the Adenoviruses as was the calculation of the % MMI. For the MMI tests, the test antigen concentrations were 30 µg/ml for the virions and 10 µgm/ml for polyhedral protein and PIBs.

## RESULTS

Morphology. Analysis of electron micrographs of intact polyhedra revealed that the two PIB types were very similar in size and shape. Both samples consisted of irregular inclusion bodies composed of numerous unsymmetrical facets, the majority of which were quite angular. A small percentage of cuboidal inclusions was found in each sample. In overall diameter, T. ni PIBs were 1.54 ± 0.04 µ; A. californica PIBs were 1.53 ± 0.03 µ.

Sectioned PIBs revealed that occlusion of virions was random for both insect pathogens. Based on over 150 observations, polyhedra of T. ni were found to contain 4.50 ± 0.11 virions per developmental membrane; A. californica polyhedra contained 4.54 ± 0.09 virions per membrane.

Bioassays. The calculated $LD_{50}$ of T. ni NPV for the fall armyworm was 122,100 PIBs/g of diet; the $LD_{50}$ of A. californica NPV for the same lepidopteran was 194,400 PIBs/g of diet. The slopes of the regression lines generated from the dose-mortality data for these viruses with the fall armyworm were quite similar. In addition, the fiducial limits of these regression lines were found to overlap considerably (Fig. 2).

With the cabbage looper, the calculated $LD_{50}$ A. californica and T. ni NPV were 61.9 and 458.2 PIBs/g of diet, respectively. The slopes and fiducial limits of the regression lines generated from data with the cabbage looper were noticeably different for the two virus species (Fig. 3).

Physical data. Following isopycnic density gradient centrifugation in CsCl, the refractive index of the 10-drop fraction that contained the highest titer of PIBs was measured. The T. ni optimal fraction was found to band in CsCl at a density of 1.3104 g/cm$^3$; the buoyant density of A. californica was 1.3038 g/cm$^3$.

The two viruses were similar in the amount of polyhedral and virion protein per inclusion body (Table 10).

Macrophage migration inhibition. In all guinea pigs sensitized with 60 µg of either T. ni or A. californica virions, complete inhibition of peritoneal exudate cells occurred with 30 µg/ml of the homologous antigen. Peritoneal exudate cells from guinea pigs sensitized to T. ni virions were inhibited, when incubated with 30 µg/ml A. californica virions, 19-44% (mean inhibition, 29.4%; standard error, 3.15) compared to the migration of sensitized cells in the absence of antigens (Fig. 4). Similarly, peritoneal exudate cells from guinea

pigs sensitized to A. californica virions were inhibited 9-46% (mean, 28%; standard error, 4.07) when incubated with 30 µg/ml T. ni virions. The migration inhibition that occurred with heterologous virions was significantly different, at the 0.001 level, from that in the presence of homologous virions and in the absence of any antigens. Peritoneal exudate cells from non-sensitized guinea pigs demonstrated similar migration patterns with or without virion antigens present (Table 11).

Peritoneal exudate cells of non-sensitized guinea pigs demonstrated 100% MMI in the presence of 10 µg/ml T. ni or A. californica polyhedral protein or intact PIBs. Currently, attempts are being made to characterize and eliminate this apparently non-specific migration inhibition factor so that any antigenic differences in the protein(s) of the PIBs of these insect pathogens can be determined.

## DISCUSSION

Following passage through the fall armyworm, T. ni and A. californica NPV were virtually indistinguishable morphologically. A comparison of these viruses based on dose-mortality bioassays in the fall armyworm and cabbage looper revealed that the insect host employed was very critical in terms of distinguishing these agents. Although the cabbage looper was found to possess a much more differential susceptibility to these pathogens than the fall armyworm, another, preferably unrelated means of distinguishing these viruses was desired.

Peritoneal exudate cells from T. ni or A. californica virion sensitized guinea pigs were completely inhibited from migrating in cell media by the homologous virion antigen, but only partially by the heterologous virion antigen. Applied mechanistically to the model for MMI these results imply that, in the peritoneal exudate, sensitized lymphocytes can differentially recognize virions of the type used in sensitization from virions of another nuclear polyhedrosis virus. This recognition results in the release of a macrophage migration inhibition factor (MIF) which interacts with macrophages in the peritoneal exudate to cause MMI. Apparently, the degree of recognition (and subsequent amount of MIF released) of the challenge virions by sensitized lymphocytes is directly related to the degree of antigenic similarity between the sensitizing and challenge antigens.

A possible common antigenic component possessed by both T. ni and A. californica virions may be indicated by the similarity in the percents of MMI in the heterologous virion antigen challenges. If this were true, it would implicate the existence of a multi-component antigenic recognition in the homologous systems.

Although the MMI test has been demonstrated to be an effective means of identifying otherwise closely related nuclear polyhedrosis viruses based on differences in the antigenicity of their occluded virions, several variable factors were found to exist in the method. These included the lack of uniform sensitization, the occasional total absence of migration by macrophages, and

the variable migration of control cells, of the same animal, from one tissue culture dish to another. While these factors did not impair the essential findings of this investigation, they did necessitate the testing of an increased number of guinea pigs.

We have begun our studies of the *in vitro* passive transfer of delayed hypersensitivity to the viral antigens that have been utilized in the previous work of this project. A hot phenol extraction procedure has been modified so that highly reproducible yields of guinea pig spleen RNA are obtained. Comparative studies on the RNA content of both freshly excised spleens and stored spleens have revealed that our stored spleens yield approximately 20-25% of the RNA obtained from fresh spleens. Attempts to transfer sensitivity to normal peritoneal exudate cells with RNA extracts of spleens obtained from four animals previously shown to be sensitive to either Adenovirus 1 or *A. californica* virions by MMI tests were unsuccessful. Toxicity of the RNA preparation, a problem occasionally experienced by others utilizing this test system, was responsible for the lack of transfer of sensitivity in two instances. We are currently setting up positive controls for RNA transfer of sensitivity utilizing the spleens of animals sensitive to PPD, an antigen which has been used successfully in this type of test system by many investigators. Through MMI data on the RNA from these spleens and comparative sucrose density gradient analysis of RNA preparations, we should be able to determine if either prolonged storage under the conditions we have used or the degradation of RNA during the extraction procedure is responsible for our inability to transfer sensitivity.

## PUBLICATIONS


1. Differentiation of Viruses by Macrophage Migration Inhibition Tests. D.P. Woodman and F.M. Hetrick. Can. J. Microb. (submitted for publication)

2. Differentation of *Trichoplusia ni* MEV and *Autographa californica* MEV by Macrophage Migration Test. J. Invertebrate Path. (accepted for (publication)

3. Comparison of Neutralization and Macrophage Migration Inhibition Tests for Differentiating Adenoviruses. J.F. Novotny and F.M. Hetrick, in preparation.

Table 1

Macrophage Migration Inhibition of Guinea Pig Peritoneal Exudate Cells Sensitized with Adenovirus Type 1 Antigen

| Guinea pig | Sensitizing antigen | Test antigen | % MMI | Guinea pig | Sensitizing antigen | Test antigen | % MMI |
|---|---|---|---|---|---|---|---|
| 1 | Ad 1 70 ug | Ad 1 | 89 | 8 | Ad 1 70 ug | Ad 1 | 19 |
| | | 4 | 30 | | | 4 | 0 |
| | | 5 | 21 | | | 5 | 0 |
| | | 7 | 5 | | | 7 | 0 |
| 2 | Ad 1 70 ug | Ad 1 | 83 | 18 | Ad 1 120 ug | Ad 1 | 34 |
| | | 4 | 31 | | | 4 | 19 |
| | | 5 | 19 | | | 5 | 0 |
| | | 7 | 10 | | | 7 | 0 |
| 5 | Ad 1 70 ug | Ad 1 | 42 | 19 | Ad 1 120 ug | Ad 1 | 92 |
| | | 4 | 0 | | | 4 | 20 |
| | | 5 | 0 | | | 5 | 15 |
| | | 7 | 0 | | | 7 | 7 |

Table 2

Macrophage Migration Inhibition of Guinea Pig Peritoneal Exudate Cells Sensitized with Adenovirus Type 4 Antigen

| Guinea pig | Sensitizing antigen | Test antigen | % MMI | Guinea pig | Sensitizing antigen | Test antigen | % MMI |
|---|---|---|---|---|---|---|---|
| 11 | Ad 4 110 ug | Ad 1 | 24 | 15 | Ad 4 110 ug | Ad 1 | 0 |
| | | 4 | 39 | | | 4 | 23 |
| | | 5 | 53 | | | 5 | 0 |
| | | 7 | 0 | | | 7 | 0 |
| 12 | Ad 4 110 ug | Ad 1 | 24 | 16 | Ad 4 110 ug | Ad 1 | 0 |
| | | 4 | 82 | | | 4 | 27 |
| | | 5 | 30 | | | 5 | 6 |
| | | 7 | 32 | | | 7 | 0 |
| 13 | Ad 4 110 ug | Ad 1 | 0 | 17 | Ad 4 110 ug | Ad 1 | 59 |
| | | 4 | 66 | | | 4 | 94 |
| | | 5 | 0 | | | 5 | 54 |
| | | 7 | 0 | | | 7 | 11 |

Table 3

Macrophage Migration Inhibition of Guinea Pig Peritoneal Exudate Cells Sensitized with Adenovirus Type 5 Antigen

| Guinea pig | Sensitizing antigen | Test antigen | % MMI | Guinea pig | Sensitizing antigen | Test antigen | % MMI |
|---|---|---|---|---|---|---|---|
| 20 | Ad 5 110 ug | Ad 1 | 19 | 24 | Ad 5 110 ug | Ad 1 | 38 |
| | | 4 | 12 | | | 4 | 14 |
| | | 5 | 52 | | | 5 | 86 |
| | | 7 | 19 | | | 7 | 0 |
| 21 | Ad 5 110 ug | Ad 1 | 0 | 25 | Ad 5 110 ug | Ad 1 | 10 |
| | | 4 | 0 | | | 4 | 15 |
| | | 5 | 40 | | | 5 | 48 |
| | | 7 | 0 | | | 7 | 15 |
| 22 | Ad 5 110 ug | Ad 1 | 0 | 26 | Ad 5 110 ug | Ad 1 | 0 |
| | | 4 | 0 | | | 4 | 0 |
| | | 5 | 99.4 | | | 5 | 49 |
| | | 7 | 33 | | | 7 | 0 |

Table 4

Macrophage Migration Inhibition of Guinea Pig Peritoneal Exudate Cells Sensitized with Adenovirus Type 7 Antigen

| Guinea pig | Sensitizing antigen | Test antigen | % MMI | Guinea pig | Sensitizing antigen | Test antigen | % MMI |
|---|---|---|---|---|---|---|---|
| 23 | Ad 7 120 ug | Ad 1 | 53 | 30 | Ad 7 120 ug | Ad 1 | 0 |
| | | 4 | 30 | | | 4 | 13 |
| | | 5 | 61 | | | 5 | 0 |
| | | 7 | 81 | | | 7 | 65 |
| 28 | Ad 7 120 ug | Ad 1 | 3 | 31 | Ad 7 120 ug | Ad 1 | 0 |
| | | 4 | 14 | | | 4 | 17 |
| | | 5 | 3 | | | 5 | 0 |
| | | 7 | 76 | | | 7 | 71 |
| 29 | Ad 7 120 ug | Ad 1 | 0 | | | | |
| | | 4 | 0 | | | | |
| | | 5 | 0 | | | | |
| | | 7 | 61 | | | | |

Table 5

Macrophage Migration Inhibition of Guinea Pig Strain 13 Histocompatible Peritoneal Exudate Cells Sensitized with Adenovirus Type 1 Antigen

| Guinea pig | Sensitizing antigen | Test antigen | % MMI | Guinea pig | Sensitizing antigen | Test antigen | % MMI |
|---|---|---|---|---|---|---|---|
| 13/1 | 100 ug Ad 1 | Ad 1 | 16 | 13/3 | 100 ug Ad 1 | Ad 1 | 24 |
| | | 4 | 27 | | | 4 | 18 |
| | | 5 | 27 | | | 5 | 12 |
| | | 7 | 25 | | | 7 | 13 |
| 13/2 | 100 ug Ad 1 | Ad 1 | 53 | 13/1, 2, 3 Pooled cells | 100 ug Ad 1 | Ad 1 | 30 |
| | | 4 | 32 | | | 4 | 22 |
| | | 5 | 12 | | | 5 | 17 |
| | | 7 | 34 | | | 7 | 17 |

Table 6. Analysis of Variance

Adenovirus Types 1, 4, 5, and 7

% MMI Results

| Group | Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F |
|---|---|---|---|---|---|
| Adenovirus 1 70 µg | Between Antigens | 3 | 7726 | 2575 | 6.36 |
| | Within Antigens | 12 | 4865 | 405 | P<.01 |
| | Total | 15 | | | |
| Adenovirus 1 120 µg | Between Antigens | 3 | 4465 | 1488 | 9.79 |
| | Within Antigens | 4 | 1819 | 152 | P<.05 |
| | Total | 7 | | | |
| Adenovirus 4 | Between Antigens | 3 | 7665 | 2555 | 5.02 |
| | Within Antigens | 20 | 10181 | 509 | P<.05 |
| | Total | 23 | | | |
| Adenovirus 5 | Between Antigens | 3 | 12390 | 4130 | 19.9 |
| | Within Antigens | 24 | 5796 | 207 | P<.01 |
| | Total | 27 | | | |
| Adenovirus 7 | Between Antigens | 3 | 12590 | 4197 | 11.53 |
| | Within Antigens | 16 | 5822 | 364 | P<.01 |
| | Total | 19 | | | |
| Adenovirus 1 Strain 13 Guinea Pigs | Between Antigens | 3 | 396 | 132 | 1.24 |
| | Within Antigens | 12 | 1276 | 106 | P>.05 |
| | Total | 15 | | | |

Table 7

Macrophage Migration Inhibition of Peritoneal Exudate Cells from Guinea Pigs Sensitized with Increasing Concentrations of Antigen

| Guinea pig | ug Ad 1 sensitizing antigen | % MMI Test Antigens | | | |
|---|---|---|---|---|---|
| | | Ad 1 | Ad 4 | Ad 5 | Ad 7 |
| 25/1 | 25 | 0 | 0 | 11 | 11 |
| 25/2 | | 5 | 20 | 1 | 15 |
| 25/3 | | 11 | 0 | 0 | 17 |
| 25/4 | | 0 | 8 | 0 | 0 |
| 25/5 | | 0 | 0 | 13 | 0 |
| 50/1 | 50 | 43 | 28 | 22 | 44 |
| 50/2 | | 31 | 0 | 0 | 38 |
| 50/3 | | 0 | 0 | 0 | 0 |
| 50/4 | | 22 | 0 | 0 | 26 |
| 100/1 | 100 | 74 | 21 | 3 | 0 |
| 100/2 | | 64 | 26 | 2 | 24 |
| 100/3 | | 74 | 28 | - | 39 |
| 100/4 | | 33 | 0 | 0 | 0 |
| 100/A | | 48 | 34 | 0 | 0 |
| 100/B | | 38 | 3 | 0 | 0 |

Table 8

Macrophage Migration Inhibition of Peritoneal Exudate Cells from Guinea Pigs Sensitized with Increasing Concentrations of Antigen

| Guinea pig | ug Ad 1 sensitizing antigen | % MMI Test Antigens | | | |
|---|---|---|---|---|---|
| | | Ad 1 | Ad 4 | Ad 5 | Ad 7 |
| 150/D | 150 | 58 | 31 | 36 | 40 |
| 150/E | | 65 | 25 | 26 | 26 |
| 200/F | 200 | 34 | 32 | 25 | 46 |
| 200/G | | 55 | 51 | 45 | 62 |
| 250/1 | 250 | 8 | 0 | 0 | 0 |
| 250/2 | | 22 | 11 | 31 | 13 |
| 250/4 | | 31 | 0 | 3 | 28 |
| 250/5 | | 0 | 0 | 3 | 4 |
| 250/6 | | 0 | 0 | 0 | 0 |
| 250/H | | 40 | 69 | 32 | 76 |
| 250/I | | 30 | 69 | 27 | 36 |

**Table 9. Analysis of Variance**

**Adenovirus Type 1 Sensitizing Dose**

**Response Data**

| Group | Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F |
|---|---|---|---|---|---|
| Adenovirus 1 | Between Antigens | 3 | 26 | 8 | 0.15 |
| 25 µg | Within Antigens | 12 | 833 | 52 | P>.05 |
| | Total | 15 | | | |
| | Between Antigens | 3 | 1482 | 494 | 2.00 |
| 50 µg | Within Antigens | 12 | 2963 | 247 | P>.05 |
| | Total | 15 | | | |
| | Between Antigens | 3 | 10136 | 3379 | 337.9 |
| 100 µg | Within Antigens | 20 | 4044 | 10 | P<.01 |
| | Total | 23 | | | |
| | Between Antigens | 3 | 1452 | 484 | 10.0 |
| 150 µg | Within Antigens | 4 | 191 | 48 | P<.01 |
| | Total | 7 | | | |
| | Between Antigens | 3 | 256 | 85 | 0.47 |
| 200 µg | Within Antigens | 4 | 728 | 182 | P>.05 |
| | Total | 7 | | | |
| | Between Antigens | 3 | 315 | 105 | 0.15 |
| 250 µg | Within Antigens | 24 | 16336 | 681 | P>.05 |
| | Total | 27 | | | |

Table 10

Protein Content of *T. ni* and *A. californica* Polyhedra

| Virus | Sample no. | Titer PIBs/ml x $10^7$ | Protein content µg/ml polyhedral | Protein content µg/ml virion | Protein content µg/PIB x $10^{-6}$ polyhedral | Protein content µg/PIB x $10^{-6}$ virion |
|---|---|---|---|---|---|---|
| *T. ni* MEV | 1 | 0.94 | 60 | 40 | 6.37 | 4.26 |
| | 2 | 1.40 | 84 | 63 | 6.00 | 4.50 |
| | 3 | 2.11 | 141 | 102 | 6.71 | 4.86 |
| *A. cal.* MEV | 1 | 1.79 | 120 | 69 | 6.66 | 3.83 |
| | 2 | 2.18 | 137 | 77 | 6.23 | 3.50 |
| | 3 | 1.23 | 82 | 50 | 6.83 | 4.17 |

Table 11

The Relative Percent Macrophage Migration Inhibition of Peritoneal Exudate Cells Challenged with T. ni and A. californica Virions Following Sensitization of Guinea Pigs with Virions

| Pig no. | Sensitization 60 μg/pig | % MMI | | |
|---|---|---|---|---|
| | | T. ni MEV[a] | A. cal. MEV[a] | Control[b] |
| 302 | T. ni MEV | 100 | 29 | 0 |
| 303 | | 100 | 44 | 0 |
| 304 | | 100 | 29 | 0 |
| 307 | | 100 | 23 | 0 |
| 308 | | 100 | 36 | 0 |
| 310 | | 100 | 19 | 0 |
| 312 | | 100 | 26 | 0 |
| 403 | A. cal. MEV | 9 | 100 | 0 |
| 405 | | 24 | 100 | 0 |
| 406 | | 33 | 100 | 0 |
| 407 | | 19 | 100 | 0 |
| 408 | | 37 | 100 | 0 |
| 409 | | 24 | 100 | 0 |
| 411 | | 46 | 100 | 0 |
| 412 | | 32 | 100 | 0 |
| 502 | CFA[c] | 0[d] | 0[d] | 0 |
| 511 | | 0 | 0 | 0 |
| 513 | | 0 | 0 | 0 |

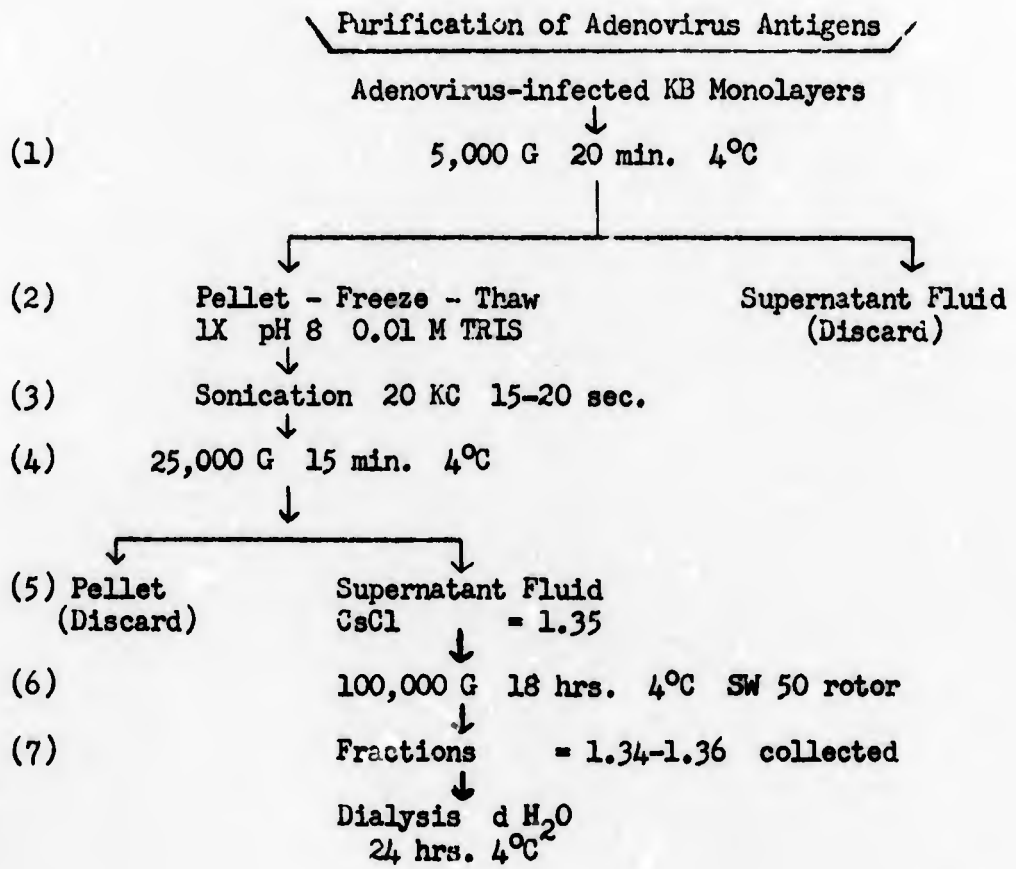
[a] 30 μg antigen/ml $EBME_{85}GP_{15}$.

[b] $EBME_{85}GP_{15}$ without antigen.

[c] Complete Freund's adjuvant.

[d] Migration greater than that in control cells without antigen is recorded as 0% MMI.

Figure 1


Purification of Adenovirus Antigens
Adenovirus-infected KB Monolayers
(1) 5,000 G 20 min. 4°C
(2) Pellet - Freeze - Thaw
1X pH 8 0.01 M TRIS
Supernatant Fluid
(Discard)
(3) Sonication 20 KC 15-20 sec.
(4) 25,000 G 15 min. 4°C
(5) Pellet
(Discard)
Supernatant Fluid
CsCl = 1.35
(6) 100,000 G 18 hrs. 4°C SW 50 rotor
(7) Fractions = 1.34-1.36 collected
Dialysis d H2O
24 hrs. 4°C

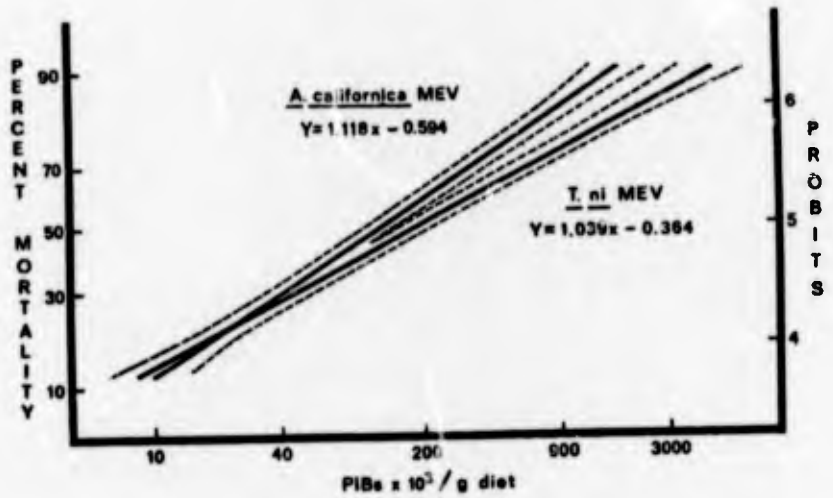



Figure 2. Dose-mortality regression lines of T. ni and A. californica NPV in the fall armyworm. Only extreme fiducial limits are represented at levels of mortality less than 40% due to overlapping of common limits.

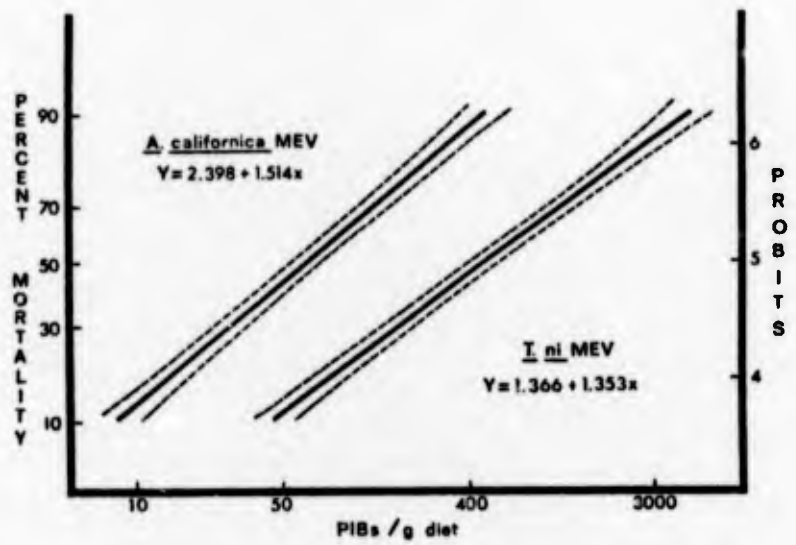



Figure 3. Dose-mortality regression lines of T. ni and A. californica NPV in the cabbage looper.

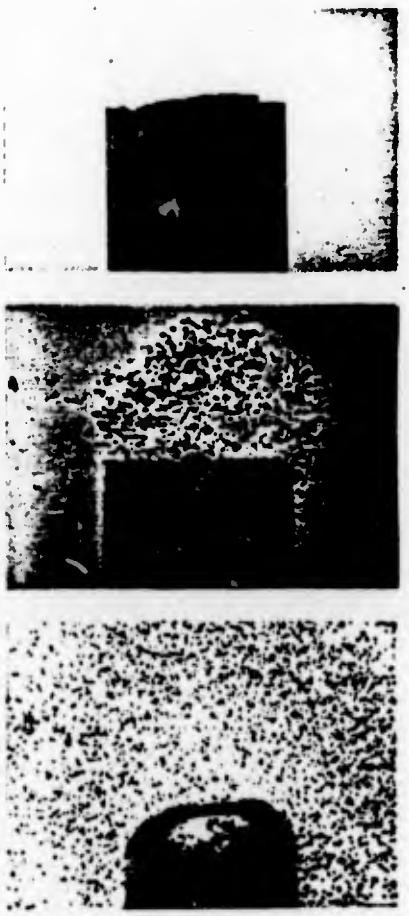


Figure 4. Macrophage migration inhibition of peritoneal exudate cells from a guinea pig sensitized to T. ni virions.

A. 100% MMI in the presence of 30 μg/ml T. ni virions.

B. 44% MMI in the presence of 30 μg/ml A. californica virions.

C. 0% MMI in the absence of antigens.

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