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Study of Delayed Hypersensitivity to Myxoviruses Induced by Vaccines

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The delayed hypersensitivity response of guinea pigs to Bacillus Calmette-Guérin (BCG) and myxovirus vaccines was investigated by use of the techniques of skin testing and inhibition of macrophage migration. A serum antibody response to the injected vaccines was readily demonstrable. Parainfluenza type 2 virus consistently failed to induce a delayed hypersensitivity state in 15 animals, even with the use of a virus adjuvant emulsion. Respiratory syncytial virus occupied an intermediate position in that positive delayed type skin tests of an erythematous nature were elicited following inoculation, but only two of seven guinea pigs yielded a positive migration inhibition test. In mumps-inoculated animals, skin testing gave rise to erythematous delayed skin reactions which varied from 0 to 20 mm in size. Migration inhibition could be demonstrated in 7 of 21 animals. In almost all guinea pigs inoculated with BCG, large, indurated, erythematous skin reactions were elicited, and inhibition of macrophage migration was readily demonstrated. The degree of skin reactivity was positively correlated with inhibition of macrophage migration. If the skin reaction to a specific antigen exceeded 9 mm of erythema, that antigen also inhibited macrophage migration. Skin testing proved to be the most sensitive indicator of viral hypersensitivity. Migration inhibition was demonstrated only when a greater than 8-mm skin reaction was evoked. This cellular hypersensitivity appeared to be a qualitative phenomenon, the expression of which could be heightened by the use of adjuvant. The applicability and sensitivity of the migration inhibition technique is considered relative to its use for in vitro monitoring of effects of viral vaccine inoculations.

In recent years, in vitro techniques have found greater applicability with respect to the determination of delayed hypersensitivity. Blast transformation of sensitized lymphocytes (11, 12) and inhibition of macrophage migration (1, 3) have received the widest attention. Most investigation with these techniques has been to elucidate the precise mechanism of the delayed hypersensitivity reaction in these in vitro models with subsequent application to human beings. Tuberculin sensitivity and the response to semisynthetic proteins have received the most study (3, 4). Little information is available with respect to hypersensitivity to viral antigens after infection with live virus or immunization with inactivated viruses.

Among the viruses, vaccinia (7), herpes (13), and mumps (6, 9) have been discussed most often with regard to the development of hypersensitivity. Experimental data to support and clarify the role of hypersensitivity to these viruses are fragmentary and generally speculative. Recent reports of adverse reactions with virus vaccines have added impetus to the need for study of the immunological mechanisms in these responses (2, 8, 15). With the in vitro technique of migration inhibition of sensitized cells (1, 3), we have investigated the delayed hypersensitivity response of guinea pigs to myxovirus vaccine inoculations. Respiratory syncytial (RS), parainfluenza type 2, and mumps viruses were evaluated with respect to their ability to induce cellular hypersensitivity with and without adjuvants and by various routes of immunization.

MATERIALS AND METHODS

Animals. Albino guinea pigs weighing 300 to 500 g were used in all of the investigations.

Viruses. Formalin-inactivated parainfluenza type 2 virus with a hemagglutination (HA) titer of 1:128 was given to guinea pigs. The virus had been passed nine times in monkey kidney tissue culture and then 60 times in eggs. Parainfluenza type 2 virus from a person with a common cold was used for skin testing and laboratory studies. It has been passed seven times in

rhesus monkey kidney tissue culture (RMK) and had an infectivity titer of 10^5/ml in RMK.

RS virus, Simon strain, was the 25th passage in bovine kidney tissue culture (BKTC) with an infectivity titer of $10^{5.5}$/ml in BKTC. Live RS virus of this harvest was employed in the laboratory studies and skin testing. For use in immunization, a sample of the virus pool was inactivated in Formalin and dialyzed.

Viable mumps virus, RiAm strain, in the 16th allantoic passage was used for immunization. The infectivity titer in eggs was 10^5/ml. The same harvest was adapted to RMK to produce the antigen used for the in vitro studies. The harvest from the fourth RMK passage, which was used, had an infectivity titer of 10^5/ml.

Immunization. Two or three inoculations of each viral antigen were given at 3-week intervals. Immunization against parainfluenza type 2 virus was by use of a formalinized chorioallantoic harvest given subcutaneously either alone or as a 1:1 emulsion with complete Freund's adjuvant. Each inoculum contained 32 to 64 HA units. In addition, live parainfluenza type 2 (MK_7) was administered intranasally and intraperitoneally to four animals. The inoculum contained 10^4 to 10^5 $TCID_{50}$. All injections of RS virus were given subcutaneously as a 1:1 emulsion of inactivated virus with complete Freund's adjuvant. Each inoculation contained 10^5 $TCID_{50}$ titrated in BKTC.

Mumps virus was used for immunization only as live virus in an allantoic fluid harvest. Each inoculum contained 10^5 $TCID_{50}$ and was administered to four animals intranasally or subcutaneously. It was also combined with complete Freund's adjuvant and given to 17 animals. In BCG-immunized animals, 1 mg of the tubercle bacillus vaccine was emulsified in complete Freund's adjuvant and injected into the foot pads.

As an index of the adequacy of the antigenic stimulus, the serum titer of antibody against the injected virus was measured at the time the animals were killed. The tests were for complement-fixing antibody against RS and mumps virus and for neutralizing antibody against parainfluenza type 2 virus.

Skin test. Skin testing of the guinea pigs was performed about 2 weeks after the last immunization—48 to 72 hr prior to death. The test viral and control antigens in a volume of 0.1 ml were injected intradermally on the abdomen after it had been shaved. The skin test sites were observed for erythema and induration. The results were recorded 24 and 48 hr after the injection. The viral and the purified protein derivative (PPD) skin test antigens consisted of parainfluenza type 2 (MK_7, 10^4/0.1 ml), RS (BK_{25}, $10^{1.5}$/0.1 ml), mumps (MK_4, 10^5/0.1 ml), and 0.00025 mg of PPD per ml. Values in parentheses refer to the number of passages on primary bovine kidney or monkey kidney cell culture and the infectious titer of the harvest. Control solutions injected were monkey kidney tissue culture fluid harvest and Earle's balanced salt solution (BSS), both of which contained penicillin and streptomycin each at 200 μg/ml.

Representative biopsies were taken of the mumps-positive skin test, the tuberculin reaction, and normal guinea pig skin. After Formalin fixation, the biopsy specimens were imbedded in paraffin, and sections were cut and stained with hematoxylin and eosin. These biopsy samples were evaluated microscopically with respect to tissue abnormalities and cellular infiltration.

In vitro test for hypersensitivity by macrophage migration inhibition. The in vitro measurement of delayed hypersensitivity was made by use of the technique of migration inhibition of sensitized peritoneal exudate cells. Guinea pigs were injected intraperitoneally with 30 cm^3 of sterile paraffin oil. After 3 days, the peritoneal exudate cells were collected, washed twice in BSS, and resuspended in medium 199 containing 1% glutamine and 15% normal guinea pig serum. The cells were packed in capillary tubes by centrifugation at 500 $\times$ g for 2 min. The tube was then broken at the interface of the cells with the fluid medium and the cell button was anchored with silicone grease in a Sykes-Moore culture chamber. Each chamber was filled with maintenance medium (medium 199, 1% glutamine, and 15% homologous guinea pig serum) to which specific viral antigens had been added. The concentrations of viral antigens in the chamber fluid were 2×10^4 $TCID_{50}$ of parainfluenza type 2 (MK_7), $2 \times 10^{4.5}$ $TCID_{50}$ of RS (BK_{25}), and 2×10^5 $TCID_{50}$ of mumps (MK_4); 12.5 μg of PPD was used. Each antigen was always tested in duplicate. After incubation at 37 C, the extent of migration was observed at 24, 48, and 72 hr, and the degree of inhibition by the test antigen was recorded as 0 to 4+. A migration inhibition test was considered positive when the radius of migration of sensitized cells in the presence of antigen was less than 50% that of cells from the same harvest without viral antigen. In control cultures and in the presence of heterologous antigens not used to immunize the animal, sufficient migration of cells generally occurred within 24 to 48 hr, at which time the differences between the test and control chambers were scored.

RESULTS

Skin testing of unimmunized guinea pigs with the viral antigens yielded negative results. Rarely, an erythematous skin reaction of less than 4 mm in size and of brief duration was observed. In 20 of 22 BCG-immunized animals, intradermal inoculation of PPD consistently evoked skin reactions in excess of 10 mm; induration and necrosis were prominent features of hypersensitivity to tuberculin.

Guinea pigs immunized with viruses exhibited a variable response to skin testing with viral antigens. All reactions that occurred were of the delayed type, i.e., after 16 to 24 hr, but none showed the degree of reactivity seen with the tuberculin reactions. When adjuvant was omitted from the immunizing inoculum, mumps and parainfluenza viruses failed to elicit a positive skin test in excess of 4 mm. The use of adjuvant augmented the response appreciably, but in 11

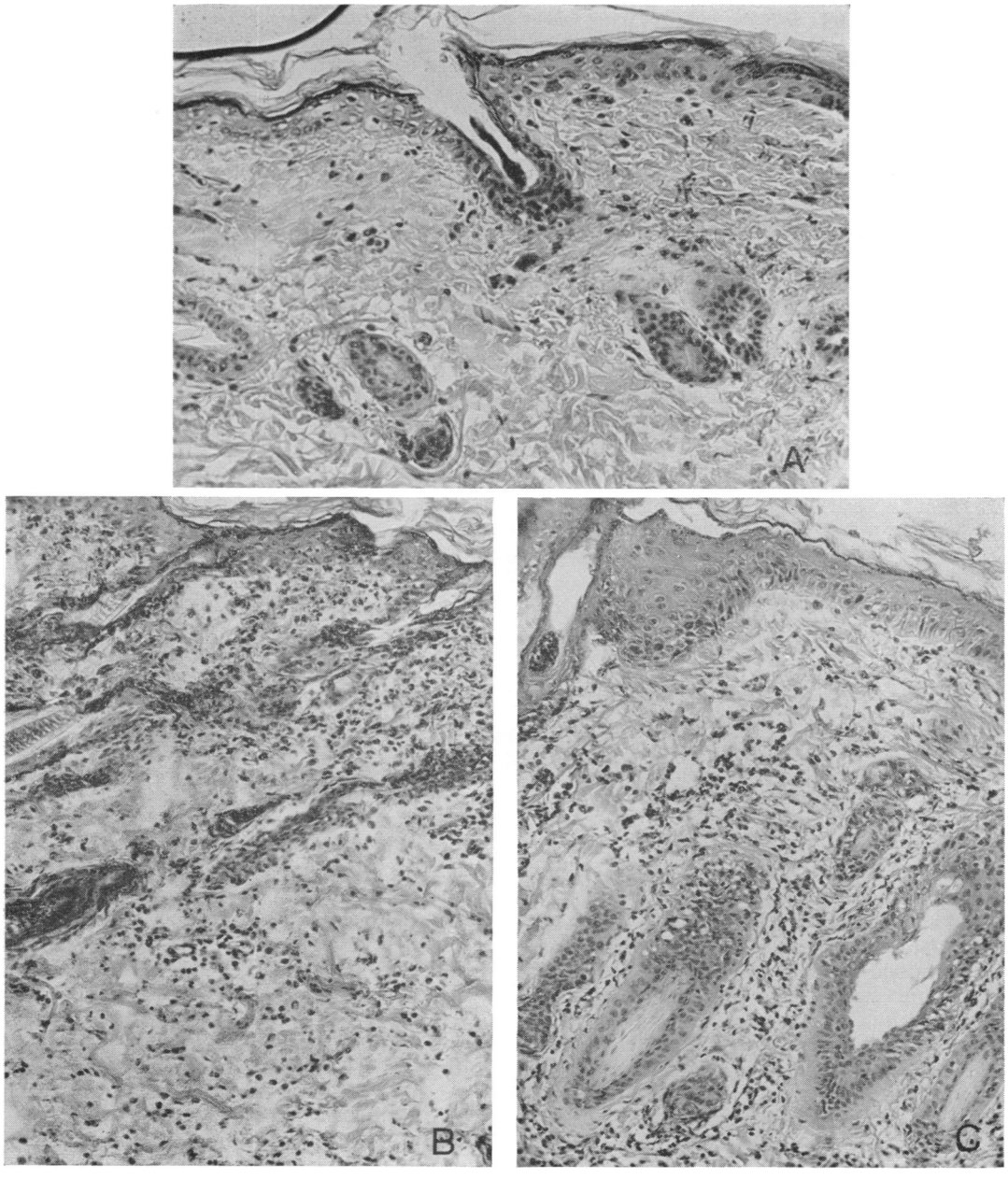

FIG. 1. *Biopsies of skin test sites after 24 hr; A, control; B, mumps; and C, PPD.*

guinea pigs inoculated with parainfluenza virus and adjuvant, the skin reaction to that virus never exceeded 8 mm. Mumps and RS virus evoked a widely scattered response, ranging from 0 to 20 mm, in specifically sensitized animals. These skin reactions were erythematous in nature but were infrequently indurated. Most of the reactions had faded markedly by 48 hr, at a time when the tuberculin reaction was still quite intense.

In Fig. 1, the histologic pattern of a control site of normal guinea pig skin and positive tuberculin and mumps skin tests are illustrated. All biopsies were taken 24 hr after skin testing. The two positive reactions can be easily distinguished

TABLE 1. *In vitro tests for macrophage migration inhibition compared with serum antibody titer of immunized guinea pigs*

Immunization treatment		Tests positive for macrophage migration inhibition in the presence of[a]		Serum antibody titer[b]		
Antigen	Adjuvant used	Homologous antigen	Heterologous viral antigens	Parainfluenza 2 neutralizing	Mumps CF[c]	RS CF
None (control)	No		0/12	1:4 (<1:4–1:16)	<1:4	<1:8
BCG	Yes	20/22				
Parainfluenza type 2	No	0/4	0/4	1:64 (1:64–1:128)		
Parainfluenza type 2	Yes	0/11	0/11	1:56 (1:4–1:128)		
RS	Yes	2/7	0/7			1:256 (1:32–>1:256)
Mumps	No	0/4	0/4		1:128 (1:64–1:256)	
Mumps	Yes	7/17	2/17		1:164 (1:16–1:256)	

[a] Values for macrophage migration inhibition are expressed as ratios of positive tests to total tests.
[b] Values are mean serum antibody titers for specific viral antibody given; ranges of values are in parentheses.
[c] Complement-fixing antibody.

from the normal by the mononuclear-cell infiltrate in the dermis. Except for a diminution in the number of mononuclear cells and perhaps less dermal edema, the mumps reaction cannot be distinguished from that induced by tuberculin. The lesion is devoid of neutrophiles or red blood cells and, therefore, is not an Arthus reaction induced by serum antibody.

Table 1 summarizes the data from the in vitro macrophage migration inhibition tests. The number of animals in each immunization group are listed according to whether adjuvant was used in the immunization. The mean serum titers of specific viral antibody in each group also are given. The titers are significantly increased against the specific virus inoculated, which indicates the adequacy of the antigenic stimulus and the humoral immune response. Control guinea pigs had low titers of naturally acquired neutralizing antibody against parainfluenza type 2 virus (1:4 to 1:16) but no complement-fixing antibody (< 1:4) against mumps and RS viruses.

The migration of peritoneal cells from nonimmunized animals was not inhibited by any of the viral antigens or BCG. Thus, no natural hypersensitivity was found to the test antigens. Among BCG-immunized animals (BCG was the antigen used as a positive control in animals also given viruses), migration of cells was inhibited by tuberculin in 20 of 22. The two negative results occurred in animals exhibiting a runting syndrome; they also failed to develop skin reactions greater than 8 mm in diameter.

In 15 animals immunized with parainfluenza type 2 virus with or without adjuvant, migration of cells was inhibited in no instance by the presence of the specific viral antigen. On the other hand, all animals tested developed specific neutralizing antibody against parainfluenza virus type 2 in serum. Many of them also received BCG vaccine and developed a positive migration inhibition test in the presence of PPD. The antigenic response elicited by these two antigens, therefore, differed with regard to hypersensitivity and circulating antibody.

Immunization with RS virus plus adjuvant consistently induced high levels of antibody. Migration inhibition by RS virus was also observed in two of seven animals, both of which had positive delayed hypersensitivity skin tests.

In seven of 17 animals immunized with mumps virus plus adjuvant, hypersensitivity was indicated by the migration inhibition test. The inhibition of macrophage migration was usually complete at 24 hr, but cell migration frequently occurred after further incubation. Positive results were obtained with either live or ultraviolet-inactivated virus. Thus, the positive inhibition response was not caused by any cytopathogenicity owing to viral growth in the test system. In four animals, live mumps virus without adjuvant was given by either the intranasal or subcutaneous route. No inhibition of cell migration was observed, although satisfactory antibody rises were elicited. When the live virus inoculum was emulsified with adjuvant and injected subcutaneously,

only a slightly higher level of circulating complement-fixing antibody was evoked, but hypersensitivity was observed.

In two animals given live mumps virus plus adjuvant, the test was not entirely specific. Migration inhibition of cells from these animals was induced by parainfluenza as well as by mumps virus. Uninfected monkey kidney tissue culture fluid or Earle's BSS had no inhibitory effect. Skin testing of these animals with mumps antigen yielded reactions exceeding 10 mm of erythema, but skin reactions to parainfluenza virus antigen were negative, 0 and 3 mm, respectively. In the other five animals with a positive migration inhibition test with mumps virus, the reaction was specific relative to the other two antigens, parainfluenza type 2 and RS viruses. In reciprocal tests, mumps virus never inhibited migration of cells from animals immunized with the latter two viruses. Migration inhibition was not a function of the height of the serum antibody titer as shown by the failure to demonstrate inhibition by mumps virus in 10 other mumps-immunized animals with similar levels of specific serum antibody.

The relation of the in vitro inhibition of cell migration to the intensity of the skin test reaction with the same antigen is shown in Fig. 2. The results from all animals evaluated by both tests

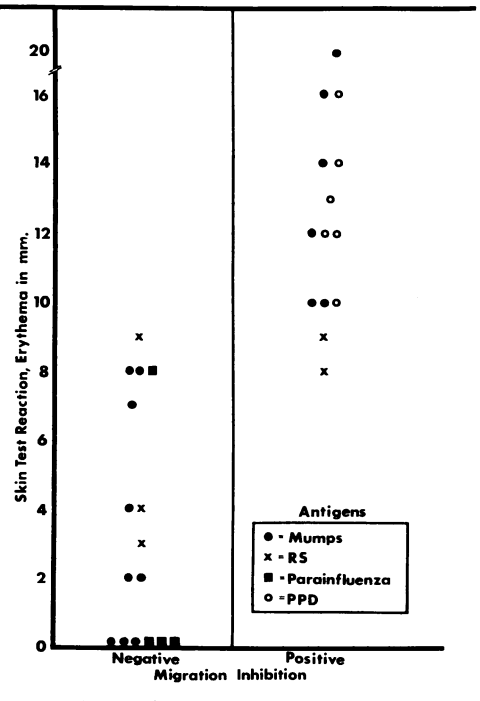

FIG. 2. *Relation of delayed hypersensitivity measured by skin test and migration inhibition.*

are noted. A clear, direct relationship can be seen. The crossover point with regard to a negative or positive migration inhibition test was a skin test reaction of 8 to 9 mm or more of erythema. BCG-immunized guinea pigs always gave strongly positive skin tests to tuberculin and also a positive migration inhibition test. Parainfluenza type 2 virus, with one exception, never elicited a positive response by either test. Mumps virus produced a full range of response by skin test, but migration inhibition occurred only when there was 10 mm or more of erythema. RS virus with adjuvant occupied an intermediate position between parainfluenza type 2 and mumps for skin test response, and migration inhibition was observed in two of three instances in which the skin reaction was 8 or 9 mm.

DISCUSSION

The role of delayed hypersensitivity in the pathogenesis of viral disease has been studied in only a few models of viral infection. Among the virulent viruses of man, vaccinia (7), measles (8), herpes (13), and mumps (6, 9) have had some investigation relative to the induction of hypersensitivity. Passive transfer experiments and viral skin testing have been the major research tools in the study of this cellular hypersensitivity because of the paucity of available in vitro techniques. Wasserman (17) observed that the addition of mumps virus to peripheral blood, after centrifugation in a capillary tube, markedly inhibited migration of the buffy coat cells. In the absence of antigen, the cells migrated well. The inhibition of migration was nonspecific, however, because cells taken from nonsensitized animals were similarly inhibited by the viral antigen. Glasgow and Morgan (9) made use of the in vitro technique employed by Rich in his original demonstration of delayed hypersensitivity in vitro (16). Spleen explants taken from control and mumps-sensitized animals were cultured in the presence of mumps viral antigen, and the extent of macrophage integrity and migration was noted. Cells taken from animals sensitized to mumps virus were consistently inhibited in their migration in the presence of mumps virus. Migration of nonsensitized cells was not affected by the viral antigen.

In our results, the intensity of delayed erythema was used as the index of hypersensitivity in the skin test. It appeared to be a valid and specific reflection of hypersensitivity. Induration could not be appreciated grossly as a part of the skin test response, but the microscopic appearance of the mumps skin reaction was entirely similar to that induced by tuberculin. It is logical that the hypersensitivity reaction proceeds from erythema

to edema and necrosis, which characterize severe delayed hypersensitivity. Severe reactions were not induced or elicited by the viral antigens. Whether this is because of the nature of the antigens or possibly related to the dose or concentration is not known. The smallest viral mass injected, as judged by infectivity titration, was parainfluenza type 2, and it caused no hypersensitivity responses. The largest infectious virus mass was the RS virus, and it caused rather meager hypersensitivity reactions even relative to mumps virus. Each of the viruses was quite antigenic with regard to serum neutralizing antibody, and the height of the titer elicited did not indicate the same order of antigenicity as did hypersensitivity. None of the viruses approached tuberculin in the regularity of induction and intensity of the hypersensitivity response. It appears, therefore, that the nature of the antigen as well as the amount or concentration are important with regard to the induction of hypersensitivity. If so, among the viruses tested, the property is greatest for mumps and least for parainfluenza type 2 virus.

It is well known that protein antigens, which oridinarily do not elicit delayed hypersensitivity when injected parenterally, acquire this ability if given as an emulsion of antigen and adjuvant (18). In our initial studies, inoculations of live and inactivated mumps and parainfluenza virus without adjuvant were given by both the intranasal and subcutaneous routes. Although we were able to elicit high antibody titers with this challenge, a delayed hypersensitivity state could not be demonstrated. Some of the mumps-immunized animals produced weak positive skin reactions, but reactions comparable to BCG were absent. With the use of virus-adjuvant mixtures, a wide spectrum of delayed hypersensitivity responses became apparent.

Sensitization to components in the supporting medium or tissue culture antigen was minimized by using a separate species of tissue culture harvest of mumps and parainfluenza virus for immunization from that used for skin testing and in vitro studies. With RS virus, bovine kidney tissue harvest was the only available material with a high titer of RS virus. The specificity for RS viral antigen in the few positive reactions observed, therefore, cannot be stated with certainty. The negative controls in the mumps and parainfluenza virus systems suggest quite strongly that the observed positive mumps responses were related to viral antigens.

By skin testing with mumps and RS viral antigens, we were able to detect cellular sensitivity to some degree in all but 3 of 24 animals immunized with the virus adjuvant emulsion. A positive skin test, however, did not insure a positive migration inhibition. Only when the skin reaction exceeded 8 mm, could we then demonstrate positive in vitro correlation to that same antigen. Migration inhibition was always less sensitive than the skin test. Similar results were noted in animal studies of delayed hypersensitivity with toxoplasmosis and Leishmaniasis (L. P. Tremonti and B. Walton, Am. J. Trop. Med. Hyg., *in press*).

Until now, the only quantitative evaluation of the macrophage migration inhibition technique has been an assay of the number of sensitized cells required to yield a positive test. David et al. (5) have shown that less than 2% of sensitized cells are necessary to elicit migration inhibitory factors. We have related the intensity of the skin reaction to migration inhibition. If a parallel can be drawn with tuberculin skin testing, the accepted norm of a 10-mm positive skin response as an indication of specific hypersensitivity has some in vitro experimental basis. Reactions greater than 10-mm skin test response indicate an order of reactivity sufficient to be demonstrable in cultures of cells.

Many animals developed circulating antibody with negative tests for hypersensitivity. This could be either failure to develop hypersensitivity or anergy. Skin test anergy has been noted in acute disseminated tuberculosis and in lepromatous leprosy. In these same diseases, the technique of blast transformation has been influenced in those instances in which autologous serum was used in the lymphocyte cultures (10; W. Bullock, Clin. Res., p. 328., 1968). It is unlikely, however, that the technique of migration inhibition, which is done with homologous serum, is influenced by serum factors developed by the antigenic stimulus. On the basis of these results, neutralizing serum antibody is the most sensitive index of an immunologic response to myxoviruses, and migration inhibition is the least.

Interpretation of the observation that cells taken from two of the mumps-immunized animals were also inhibited by parainfluenza virus is perplexing. In these animals, skin reactions to the mumps virus were intense, but those with parainfluenza were negative or slight. This was the only example of a dichotomy between the in vitro results and skin testing. It raises the possibility that the skin test and the in vitro model measure different aspects of a single immunologic mechanism. If cross-reactivity between the mumps and parainfluenza antigen is postulated, only a one-way cross was found. Although one-way cross-reactions occur with antibody, such a suggestion

has not been made previously with regard to hypersensitivity. Further experience is necessary to determine the possible importance of apparent nonspecificity in some of the tests.

If cellular hypersensitivity can be implicated in the pathogenesis of disease, the demonstration of delayed hypersensitivity induced by the inoculation of viruses raises a number of hypothetical questions. The use of adjuvants in viral vaccines could be theoretically harmful or, if the development of hypersensitivity is an essential part of immunity, it could be beneficial. Conceivably, a delayed hypersensitivity state can be induced in man by a respiratory virus vaccine without the use of adjuvant. A suggestion of this mechanism may have been seen in measles (8, 15) and RS virus vaccine trials (2). The appearance of hemorrhagic measles and severe respiratory disease when vaccinated individuals become naturally infected with these viruses raises the possibility of a vaccine-induced immune hypersensitivity response of a type not normally seen when these infections are acquired naturally. Lennon and Isacson (14) have shown the induction of delayed hypersensitivity in humans, as measured by skin testing to both measles and polio virus after vaccination with virus alone. In all studies so far, however, the intensity of the reactions to viruses has been far less than that elicited by tuberculin.

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LITERATURE CITED

1. Bennett, B., and B. Bloom. 1967. Studies on the migration inhibitory factor associated with delayed type hypersensitivity: cytodynamics and specificity. Transplantation 5:996–1001.
2. Chanock, R. M., C. B. Smith, W. T. Friedwald, R. H. Parrott, B. R. Forsyth, H. V. Coates, A. Z. Kapikian, and M. A. Gharpure. 1967. Resistance to parainfluenza and respiratory syncytial virus infection—implications for effective immunization and preliminary study of an attenuated strain of RS virus. First International Conference on Vaccines Against Viral and Rickettsial Diseases of Man. Pan American Health Organization Scientific Publication no. 147, p. 53–61.
3. David, J., S. Al Askari, H. Lawrence, and L. Thomas. 1964. Delayed hypersensitivity in vitro: the specificity of inhibition of cell migration by antigens. J. Immunol. 93:264–273.
4. David, J., H. Lawrence, and L. Thomas. 1964. Delayed hypersensitivity in vitro: the specificity of haptene protein conjugates in the inhibition of migration. J. Immunol. 93:279–282.
5. David, J., H. Lawrence, and L. Thomas. 1964. Delayed hypersensitivity in vitro. J. Immunol. 93:264–273.
6. Enders, J., L. Kane, E. Maris, and J. Slokes. 1946. Immunity in mumps. J. Exptl. Med. 84:341–364.
7. Friedman, R., S. Baron, C. Buckler, and R. Steinmuller. 1962. The role of antibody, delayed hypersensitivity, and interferon production in recovery of guinea pigs from primary infection with vaccinia virus. J. Exptl. Med. 116:347–356.
8. Fulginiti, V., and C. Kempe. 1967. Killed measles virus vaccine. Lancet 2:468.
9. Glasgow, L., and H. Morgan. 1957. Studies of delayed hypersensitivity in vitro. J. Exptl. Med. 106:45–52.
10. Heilman, D., and W. McFarland. 1966. Inhibition of tuberculin induced mitogenesis in cultures of lymphocytes from tuberculous donors. Intern. Arch. Allergy Appl. Immunol. 30:58–66.
11. Heilman, D., and W. McFarland. 1966. Mitogenic activity of bacterial fractions in lymphocyte culture. J. Immunol. 96: 988–991.
12. Hirschborn, K., F. Bach, R. Kolodny, L. Firscheen, and N. Hashem. 1963. Immune response and mitosis of human peripheral blood lymphocytes in vitro. Science 142:1185–1187.
13. Lausch, R., J. Swyers, and H. Kaufman. 1966. Hypersensitivity to herpes simplex virus. J. Immunol. 96:981–987.
14. Lennon, R., and P. Isacson. 1967. Delayed dermal hypersensitivity following killed measles vaccine. J. Pediat. 71: 525–529.
15. Rauh, L., and R. Schmidt. 1965. Measles immunization with killed virus vaccine. Am. J. Diseases Children 109:232–237.
16. Rich, A., and M. Lewis. 1932. The nature of allergy in tuberculosis as revealed by tissue culture studies. Bull. Johns Hopkins Hosp. 50:115–132.
17. Wasserman, J. 1962. Specific and non-specific inhibition of the in vitro migration of leukocytes from mumps virus-sensitized and tuberculin-sensitized guinea pigs. Acta Pathol. Microbiol Scand. 54:305–318.
18. White, R. 1967. Role of adjuvants in the production of delayed hypersensitivity. Brit. Med. Bull. 23:39–45.