

DELAYED HYPERSENSITIVITY IN MICE

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WHEREAS delayed hypersensitivity in its best-known form, tuberculin allergy, has been easy to induce and detect in guinea pigs, rabbits, man, and the ungulates, failure to detect it in mice and rats has been so common that these species generally are thought unable to develop it. The strongest point supporting this thought is that a classic delayed type of skin reaction has never been demonstrated in members of either species. Careful review of published experiments, however, reveals that rats and mice can be so sensitized and their reactions detected if special precautions and techniques are employed. A discussion of the most significant among these experiments will be presented. This discussion will be preceded by descriptions of experiments in white mice offering new evidence to supplement that of other workers and utilizing a novel technique which measures the reactions objectively where they appear to occur most intensely—in the lungs.

MATERIALS AND METHODS

CF#1 male and female white mice, 4 to 6 weeks old, were purchased for these experiments and kept in small groups. They were fed Rockland mouse pellets and water ad libitum and cracked corn and whole wheat twice a week.

Both vaccinating and challenge antigens were suspended in physiologic sodium phosphate buffer at pH 7.4. When water-in-oil (w-o) emulsions were employed for vaccination, they consisted of five parts buffer vigorously mixed with ten parts light mineral oil and two parts mannide mono-oleate (Arlacel A).

Two human varieties of tubercle bacilli, cultured on slightly modified Kirchner liquid medium, were utilized both for vaccination and for challenge. One was the avirulent strain H37Ra, whereas the other was the virulent strain Erdman. Bovine gamma globulin (Cohn Fraction II), bought from Nutritional Biochemicals Corporation, Cleveland, Ohio, was stored as a powder at 4° C. until used. Tuberculo-protein, also stored as a dried powder, was prepared from Seitz-filtered, unheated culture medium supporting growth of H37Ra bacilli by precipitation with 60 per cent w/v ammonium sulfate followed by dialysis against distilled water and then lyophilization. A crude lipid adjuvant was extracted from heat-killed Erdman bacilli with acetone and purified somewhat by evaporating the acetone and redissolving the essential lipid from the remaining aqueous residue suspension with 3 volumes of low-boiling-point petroleum ether. This solvent then was evaporated and the lipid stored in a stoppered amber bottle until used.

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Bacillary suspensions were made from ten- to fourteen-day cultures by homogenization with a fine-grade Ten Broeck tissue grinder at several times the final concentration required and then appropriately diluted. Bacillary concentrations are expressed as milligrams of blotted but moist bacilli.

Ordinarily, mice received two vaccinations one week apart and were challenged two weeks after the second, but this procedure was varied occasionally, as noted below. Challenge was by intravenous injection of 0.2 ml. phosphate-buffered bacillary suspension, considerable care being taken to ensure challenge uniformity from animal to animal. Thus, injections were made with a 0.5 ml. tuberculin syringe and 26 gauge needle, only two doses being withdrawn at one time from a frequently shaken vaccine bottle of stock suspension. Precautions also were taken to minimize nonspecific lung damage by the injected material.

At various intervals after challenge, mice were killed with ether vapor and their lung densities were determined by a technique described elsewhere.^{1, 2} Essentially, the technique consists of extracting the entire lungs from a freshly killed mouse and dropping them into each of a series of nonpolar solvent baths, progressively increasing in density by increments of 0.02 Gm. per milliliter until a density which floats them is reached. The mean of this and the density of the preceding bath is recorded as the lung's density. Whereas normal mouse lung density ranges from just below 0.68 to 0.70, lungs from mice undergoing delayed hypersensitivity reactions usually equaled or exceeded a density of 0.73 in the present experiments.

EXPERIMENTS AND RESULTS

During immunization experiments in which H37Ra-vaccinated mice were challenged intravenously with living Erdman tubercle bacilli and their lung densities were determined over the next few days to follow development of tuberculosis, attention was drawn to small but apparently significant transitory lung density increases occurring in vaccinated mice from one to three days following challenge. These increases were not seen among unvaccinated mice, and they could be distinguished from later progressive and greater lung density increases due to developing tuberculosis. An experiment set up to study the reaction sequence illustrates these observations.

Participants in one mouse group received two weekly intraperitoneal injections of 0.25 mg. living H37Ra bacilli in 0.1 ml. w-o; those in the second group were injected identically but with an emulsion containing no antigen. Both groups were challenged one week after the second injection with 0.03 mg. of living Erdman bacilli in 0.2 ml. buffer, and lung densities of three or four mice from each group were measured one, six, and twenty-four hours and two, three, four, and six days after challenge.

Results from this experiment, plotted in Fig. 1, show that during the first twenty-four hours after challenge lung densities remained normal for both groups of mice, except for a spuriously high first sampling from the immunized group. In this group, one of four mice had a lung density of 0.81, whereas

the other three had lung densities of 0.71. Later investigation showed this single high reading to have no allergic significance. Deviations of this kind did not occur among mice of any other sampling in this experiment, although they have occurred infrequently among samplings from occasional other experiments.

From Fig. 1, it is obvious that H37Ra-vaccinated mice reacted to tubercle bacilli injected intravenously with lung density increases occurring between the second and fourth days after challenge, while lung densities among w-o-vaccinated mice challenged identically remained normal until about the sixth day, when they began to increase as a manifestation of tuberculous infection. By this time, lung densities in the immunized mice had returned to the normal range.

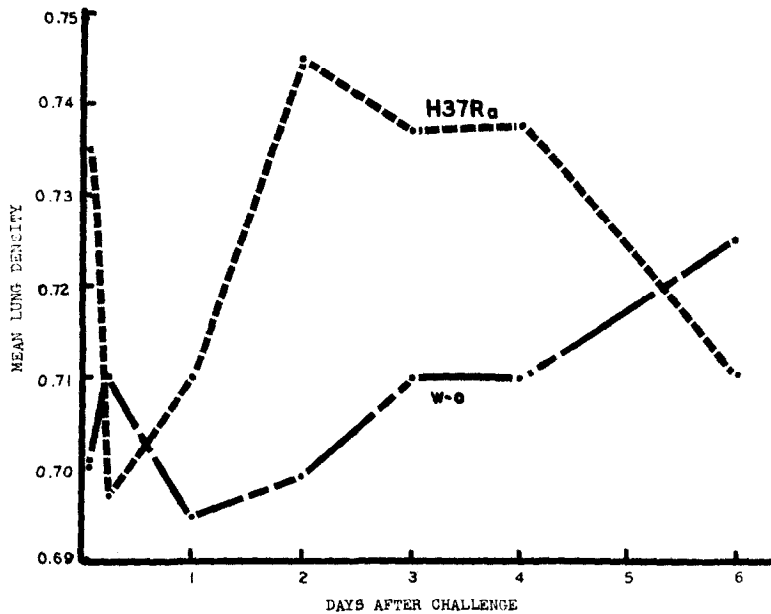


Fig. 1.—Mean lung densities of vaccinated (H37Ra) and control (w-o) mice determined at various intervals after their intravenous challenge with 0.03 mg. living tubercle bacilli (Frdman strain).

Neither group of mice gave any overt evidence of either anaphylactoid or systemic delayed hypersensitivity shocks at any time after challenge. However, whereas the lungs of challenged control mice were a light pink, characteristic of normal mouse lungs, until the last sampling, lungs from the test mice sampled two, three, and four days after challenge showed varying degrees of hemorrhagic congestion with numerous minute hemorrhagic spots scattered throughout them. A correlation appeared to exist between increased lung density and increased lung redness. Such lung pathology seems to be found commonly in tuberculin-shocked mice.^{3, 4}

This experiment was designed primarily to establish the delayed time relationship between challenge and the increased lung density reaction in sensitized mice as compared with unsensitized mice. Although virulent bacilli were

employed for challenge, the experiment suggested that the lung density reaction in sensitized mice was not due to concurrently developing infection. The frequency with which samplings were made and the consistency of findings counterbalance the sampling smallness in showing that these reactions were the

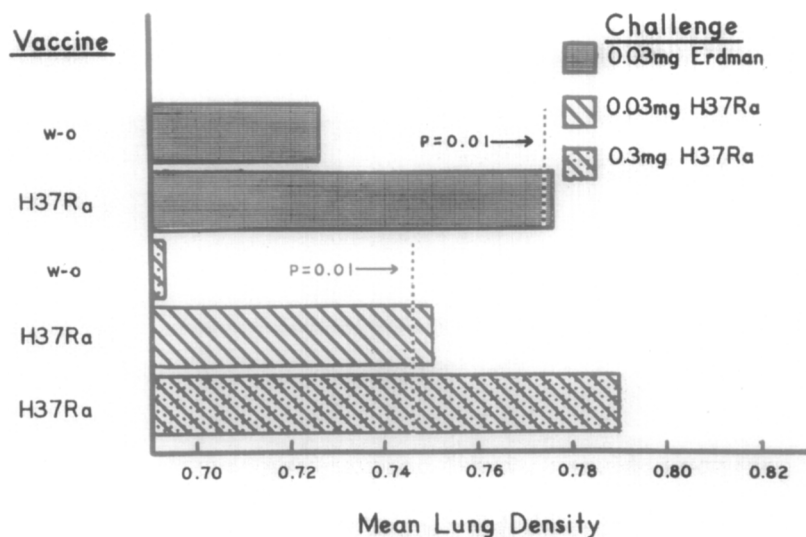


Fig. 2.—Mean lung densities of various groups of vaccinated (H37Ra) and control (w-o) mice challenged intravenously with living, virulent (Erdman) or avirulent (H37Ra) tubercle bacilli. Lung densities were determined forty-eight hours after challenge. Two different challenge doses of avirulent bacilli were employed. "P" indicates the statistical significance of the results.

effects of a common cause. To substantiate these points and to observe the effect of using a large challenge, an experiment employing five groups of seven mice each was constituted as follows:

- Group 1:* Vaccinated with control emulsion and challenged with 0.03 mg. Erdman bacilli.
- Group 2:* Vaccinated with H37Ra emulsion and challenged with 0.03 mg. Erdman bacilli.
- Group 3:* Vaccinated with control emulsion and challenged with 0.3 mg. H37Ra bacilli.
- Group 4:* Vaccinated with H37Ra emulsion and challenged with 0.03 mg. H37Ra bacilli.
- Group 5:* Vaccinated with H37Ra emulsion and challenged with 0.3 mg. H37Ra bacilli.

Two weekly intraperitoneal injections were given; each consisted of 0.1 ml. of w-o emulsion, with or without living H37Ra bacilli. The bacillary dose per injection was 0.5 mg. The mice were challenged two weeks after the second vaccination and forty-eight hours later their lung densities were determined.

As the results in Fig. 2 show, both virulent and avirulent human tubercle bacilli were equally capable of eliciting characteristic delayed lung reactions in mice vaccinated with the avirulent strain; these reactions are significant statistically. A tenfold challenge dose increase did not appear to aggravate the reaction proportionately. As in the first experiment, overt systemic tuberculin shock and anaphylactoid reaction were absent. Again, lungs from sensitized mice were found congested and hyperemic.

Despite informed opinion that vaccination with living, avirulent tubercle bacilli suspended in w-o emulsion should be a highly effective sensitizing technique in most animal species,⁵ the classic method of inducing low-grade tuberculous infection to sensitize might be more effective in mice. It has been the method most commonly used by other experimenters dealing with this problem (see discussion). The fact that mice strongly resist subcutaneous tuberculous infection⁶ suggested the practicality of an experiment containing three groups of ten mice each.

- Group 1:* Challenged intravenously with 0.03 mg. living Erdman bacilli suspended in 0.2 ml. buffer forty-eight hours before autopsy and twenty-one days after beginning of experiment.
- Group 2:* Vaccinated subcutaneously with 0.03 mg. living Erdman bacilli in 0.1 ml. buffer twenty-three days before autopsy; not subsequently challenged.
- Group 3:* Vaccinated as in Group 2 and challenged twenty-one days later, as in Group 1.

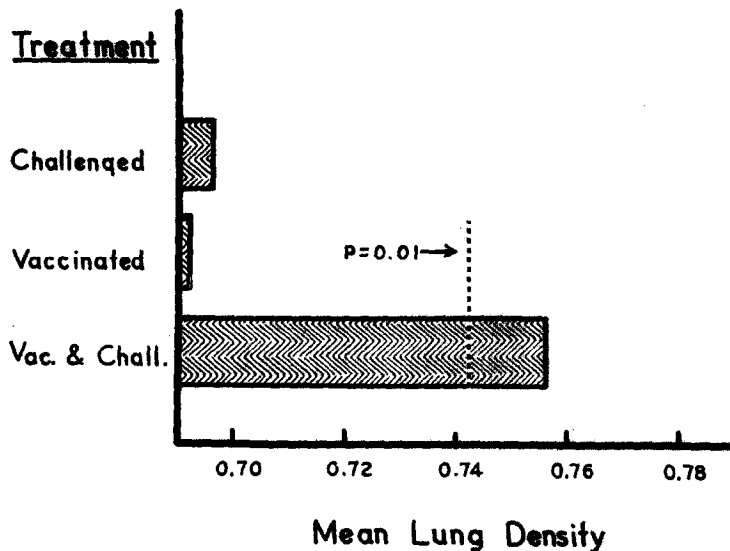


Fig. 3.—Mean lung densities of mice (1) challenged intravenously with 0.03 mg. living virulent tubercle bacilli, (2) vaccinated subcutaneously with 0.03 mg. living virulent tubercle bacilli, or (3) vaccinated subcutaneously with 0.03 mg. and then challenged intravenously with 0.03 mg. living virulent tubercle bacilli. Lung densities were determined at the same time for all three groups forty-eight hours after challenge of the upper and lower groups. "P" indicates the statistical significance of the results.

Fig. 3 shows the mean lung densities of the three groups determined at the same time in the experiment and forty-eight hours after challenge of Groups 1 and 3. Neither subcutaneous infection twenty-three days before autopsy nor intravenous infection forty-eight hours before autopsy increased mouse lung densities. Since these were the vaccinating and challenging procedures, respectively, it is obvious that the statistically significant mean lung density increase of the third mouse group is the specific consequence of challenge following vaccination. The severity of reaction was no greater than that seen in the previous two experiments. If this is an indication of the degree of hypersensitivity induced, then low-grade infection with virulent tubercle bacilli does not induce greater reactivity, under the conditions employed in these experiments, than vaccination with w-o emulsified avirulent tubercle bacilli.

The delayed lung reaction which has been the subject of these experiments appears to be specific and classifiable as an immunologic phenomenon. Evidence suggests that it is a manifestation of delayed hypersensitivity. Direct proof of this might be obtained by transferring this hypersensitivity passively from sensitized to unsensitized mice using the types of cells thought capable of this kind of transfer in other animal species but failing to do so using serum from sensitized donors alone. Preliminary attempts at cellular passive transfer with mice so far have not been successful in this laboratory. Evidence has been gathered, however, to show that the delayed lung reaction cannot be due to an immediate (anaphylactic) type of hypersensitivity.

Perhaps the most pointed reason for suspecting immediate hypersensitivity as the cause of this reaction, aside from current common knowledge that it is not difficult to induce in mice, is the fact that entire tubercle bacilli were employed as challenging antigens and that as coherent structures they could effect this reaction by (1) depositing in the lung and (2) releasing eliciting antigen only slowly into surrounding tissues. Bacilli have been employed in these experiments because they were being used when the reaction first was observed and because they seem better than tuberculin or tuberculoprotein alone for detecting delayed hypersensitivity.^{7, 8} Two overlapping approaches were made toward showing that neither a general immediate hypersensitivity reaction with delayed consequences nor an artificially delayed and localized hypersensitivity reaction was responsible for delayed lung reactions in vaccinated and challenged mice. The first approach employed active induction of immediate hypersensitivity to bovine gamma globulin (BGG) and determination of lung densities during and after anaphylaxis; the second showed that passively transferred immediate hypersensitivity to tuberculoprotein did not predispose to the delayed lung reaction following intravenous injection of either the protein or entire tubercle bacilli.

Two BGG experiments were set up. In the first, a group of mice was sensitized to BGG with two simultaneous subcutaneous injections in opposite groins of 0.1 ml. w-o emulsion containing, between them, 0.1 mg. of tuberculolipid adjuvant and 0.1 mg. of BGG. Ten days later members of this and of a second control group, vaccinated solely with emulsion, were challenged intravenously with 0.05 mg. BGG in 0.1 ml. phosphate buffer.

Whereas the control mice were unaffected by this challenge, all the test mice developed severe anaphylaxis within five to fifteen minutes, this reaction reaching a climax about thirty minutes after challenge. The mean lung densities of both groups determined at this time, during acute anaphylaxis, were essentially normal, being 0.71 and 0.67 for test and control groups, respectively. No hemorrhagic congestion was apparent; the color in both sets of lungs was normal.

In mice of this experiment combination of antigen with antibody probably was quite rapid. Since such rapid combination may not have favored manifestation of a delayed reaction, in the next experiment mice sensitized with BGG as in the first, but with H37Ra tubercle bacilli employed as adjuvant, were challenged along with unsensitized control mice with three succeeding BGG injections by different routes. This challenge procedure was calculated to achieve desensitization followed by little or no deviation of antigen from potential sites of the hypothetical delayed reaction. Such a method of challenge might have the same results as slow diffusion of antigen from tubercle bacillary bodies postulated as a possible occurrence in the previous experiments where delayed reactions were seen.

TABLE I. SUMMARY OF THREE IMMEDIATE HYPERSENSITIVITY EXPERIMENTS

EXPERIMENT	SENSITIZATION	INDUCTION PERIOD	CHALLENGE	ROUTE*	AUTOPSY: TIME AFTER CHALLENGE	MEAN LUNG DENSITY	
						TEST	CONTROL
First BGG	Active	10 days	0.05 mg. BGG	I.V.	30 minutes	0.71	0.67
Second BGG	Active	24 days	0.1 mg. BGG	S.C.	} = 48 hours	0.70	0.72
			1.0 mg. BGG	I.P.			
			1.0 mg. BGG	I.V.			
Tubercle bacillus and protein	Active	10 days	1.0 mg. protein	I.V.	48 hours	0.70	
	Passive	24 hours	1.0 mg. protein	I.V.	48 hours	0.68	
	Passive	24 hours	0.03 mg. bacilli	I.V.	48 hours	0.69	
	Passive	48 hours	0.03 mg. bacilli	I.V.	48 hours	0.69	

*I.V. = Intravenous.
I.P. = Intraperitoneal.
S.C. = Subcutaneous.

After the presence of anaphylactic hypersensitivity had been proved by preliminary challenge of a portion of the vaccinated mice, the rest and their unsensitized controls were challenged subcutaneously with 0.1 mg. BGG. This was sustained without obvious deleterious effect by both test and control mice. Twenty-four hours later members of both groups were injected intraperitoneally with 1 mg. BGG, and when this treatment also had no effect they received a third injection, one hour later, of 1 mg. BGG by the intravenous route. This final challenge, which was fatal to two of five of the same mouse group used without desensitization to prove anaphylactic sensitivity present and nearly fatal to the other three, caused no illness. Lung densities of these mice, taken forty-eight hours after the first (subcutaneous) challenge, were practically the same and nearly normal for test and control mice (Table I).

This kind of suggestive evidence that immediate hypersensitivity was not causing delayed lung reactions upon challenge was substantiated by the following experiment employing tubercle bacilli and tuberculoprotein. Twenty-five mice were vaccinated with two simultaneous subcutaneous injections, just as in the first BGG experiment, except that tuberculoprotein replaced BGG as antigen. Ten days later five of these mice were challenged intravenously with 1 mg. of tuberculoprotein. Four of the five rapidly developed severe anaphylactic shock, while the fifth showed a moderate degree of shock. All five recovered, however, and when they were autopsied two days later they exhibited normal lung densities. The remaining twenty sensitized mice were bled to death, the bloods were pooled and allowed to clot, and the resulting serum was employed for passive sensitization of a second group of twenty-five previously untreated mice.

Each recipient was injected intraperitoneally with 0.22 ml. of undiluted donor serum twenty-four and forty-eight hours before challenge, these transfers being arranged so that all mice could be challenged at the same time with the same bacillary preparation. Twenty-four hours after passive transfer, five of these mice were challenged intravenously with 1 mg. tuberculoprotein to ascertain whether passive transfer of immediate hypersensitivity had succeeded. Three of these five quickly developed strong anaphylaxis, one developed intermediate anaphylaxis, and one developed very light anaphylaxis. Since all recovered, their lung densities also were determined forty-eight hours after challenge and, as in the actively sensitized mice, were found to be normal.

Inasmuch as the remaining twenty recipients now could be considered passively sensitized to tuberculoprotein (immediate hypersensitivity), each was challenged intravenously with 0.03 mg. of H37Ra, ten twenty-four hours after passive transfer and ten forty-eight hours after transfer. Table I, which summarizes results from this and the two preceding experiments, shows that the mean lung densities of both groups were normal when determined forty-eight hours after challenge. Immediate hypersensitivity, then, does not account for delayed lung reactions detected in vaccinated mice subsequently challenged intravenously with tubercle bacilli.

DISCUSSION

Debate as to whether or not mice can develop delayed (tuberculin) hypersensitivity resembles that, now receding, concerning their competence to show anaphylactic hypersensitivity. Much evidence used in both arguments has been negative; the phenomena were thought absent because they were not detected. Just as increasingly efficient induction and detection techniques have been used to prove mice anaphylactically sensitizable, however, improved methods seem likely to verify tuberculin sensitization. Unfortunately, advances along this line of experimentation seem to have been hampered by inadequately disseminated information, due largely to the fact that most work on delayed hypersensitivity in mice or rats has been done as an accessory to tuberculosis research, thereby attracting only limited attention from researchers in other fields.

Therefore, at a time when generally held ideas on the subject are in flux, I hope that the following short review of significant experiments may stimulate wider thought and experimentation in this virtually unexplored and in many ways seemingly peculiar field, particularly with nontubercle bacillus antigens. Because available data from experiments with mice and rats appear interchangeable relative to the subject of this short review,^{6, 9-11} they will be used to supplement each other.

Although delayed hypersensitivity can be detected simply in guinea pigs, rabbits, man, and several other animal species by their reaction to tuberculin injected intradermally, this classic skin test never has been duplicated in mice or rats, even when they were proved sensitive by other techniques.^{3, 4, 7, 10} Although, with some reason, this has evoked assumptions that mice and rats do not develop delayed hypersensitivity, it is worth while to recall that conditions can be arranged under which the skin of a guinea pig with delayed hypersensitivity to dinitrochlorobenzene, for example, becomes unreactive while the guinea pig itself retains normal reactivity.¹² Therefore, although a typical positive skin test would indicate the presence of tuberculin allergy, its absence has little significance other than to suggest that sensitized mouse and rat skin reactivity is not like that of guinea pigs. For the present, then, evidence from other kinds of tests and other criteria must be considered in deciding whether or not mice and rats can develop tuberculin allergy.

Smith and Hendrick¹³ were among the first to induce and detect tuberculin shock in rats. In their studies of nutritional change effects on experimental tuberculosis in albino rats, they observed that when these animals had been infected intraperitoneally with 5 mg. of virulent human tubercle bacilli and were challenged by the same route between one and five months later with 0.5 ml. of commercial Old Tuberculin, they underwent systemic tuberculin shock. Some died, and those that recovered needed one to two days in which to do so. Interestingly, Vitamin A deficiency appeared to aggravate tuberculin shock, and adequate supplies of the vitamin seemed to diminish it.

Hehre and Freund¹⁰ skin-tested tuberculous rats with tuberculin at various intervals after intravenous infection with virulent bovine tubercle bacilli. Although antigen absorbed from the injection site provoked severe and occasionally fatal shock in some of these animals, invariably it had no effect on the skin. They were able to elicit tuberculin shock in similarly infected rats by intraperitoneal challenge and inferred from this that changing challenge routes did not influence the systemic effect of tuberculin. The amount of tuberculin they used to induce fatal shock reproducibly was large (100 to 1,000 mg.) but not harmful to uninfected animals; however, smaller amounts (20 mg.) were employed with somewhat less uniform results.

Gerstl and Thomas¹⁴ have presented analogous data for mice. Skin tests with 0.1 ml. of 0.1 per cent purified tuberculoprotein derivative in mice vaccinated intensively with living bovine or human tubercle bacilli were negative, whereas intrapleural or intratesticular tests in similarly vaccinated mice appeared to have some allergic effects. Thus, while control mice were unaffected

by either of these types of challenge, test mice developed testicular hemorrhages and tubule necrosis eighteen to twenty-four hours after challenge by that route, and mice challenged intrapleurally showed increased diffusion of lymphocytes and monocytes into the pleural cavity.

Selbie and O'Grady¹⁵ noticed that mice superinfected intramuscularly developed temporary swelling of the superinfected leg which reached its maximum twenty-four hours after challenge and then receded. Primary infection, by comparison, caused transitory swelling as a consequence of injection trauma, and this was less intense and disappeared far sooner than the swelling of superinfection. The delayed-swelling reaction regressed before subsequent inflammation due to actual infection made its appearance. Time relationships suggested to these workers that the temporary, delayed superinfection swelling reaction that they observed was a delayed hypersensitivity reaction. Skin tests on these mice, with either tuberculin or tubercle bacilli, uniformly were negative. More recent experiments published by O'Grady⁷ bear out these authors' interpretation. O'Grady describes apparent desensitization of tubercle bacilli-infected mice by repeated intramuscular Old Tuberculin injections, so that mice reacting quite obviously by delayed thigh swelling at the beginning of treatment showed only immediate transitory swelling characteristic of unsensitized mice after this course of treatment. It is instructive to note that mice infected with the larger of two test bacillary doses would not react to the muscle test until after they had been so tested twice, even though they could be shown hypersensitive by systemic tuberculin shock, or to entire tubercle bacilli injected into the muscle. Commenting on this, O'Grady points out that the ease with which tuberculin reactions can be brought about in mice depends both upon methods used and upon tissues challenged and, hence, it appears that persistently negative skin tests in these animals are no proof that the animals are not allergic to tuberculin.

As the discussion of tuberculosis pathology below will show, various mouse and rat strains probably differ remarkably in their capabilities to develop tuberculin allergy. This may explain the noteworthy success of the Gray and Jennings⁴ footpad test, a close relative of the skin test. Whereas most laboratories have employed white mice for obvious reasons of convenience, these workers have made use of the black C57 strain of mice. The generally lower resistance to tuberculous pathologic changes exhibited by this and other pigmented mouse strains^{6, 16} could be due to their becoming sensitized to tubercle bacillary constituents more readily than white mice. Striking differences even among white mice should not be unexpected, for they have been observed with regard to a very sensitive anaphylactic hypersensitivity test.¹⁷

Gray and Jennings⁴ observed that tuberculin injected into the footpad of an allergic tuberculous C57 mouse caused the foot to swell a few hours after challenge and to remain swollen for at least forty-eight hours, after which foot size slowly returned to normal. Anaphylactic sensitivity apparently played no role in this reaction, and the feet of normal mice challenged identically did

not swell appreciably. Recently, Gray and Affleck¹⁸ utilized the footpad technique for an interesting study of succeeding states of tuberculin anergy and allergy in slowly advancing mouse tuberculosis. This study suggests two often unrecognized reasons for mice and rats to develop no delayed hypersensitivity: (1) these animals may die of acute tuberculosis without having sufficient time to develop delayed hypersensitivity or its pathologic manifestations, or (2) they may be so severely infested as to remain anergic by a process where desensitization exceeds sensitization. Such difficulties as these might be minimized if avirulent or killed tubercle bacilli were used to induce allergy.

Demonstrating delayed hypersensitivity by the footpad test may be possible only in certain mouse strains. For example, Gastinel, Vaisman, and Dunoyer³ could induce severe specific tuberculin shock in chronically infected tuberculous white mice by injecting them subcutaneously with tuberculin but detected no reactions in these mice either to skin tests or to the Gray and Jennings footpad test. Selbie and O'Grady¹⁵ mentioned that their Strong A white mice which reacted to tuberculin injected intramuscularly did not react to tuberculin injected into the footpad.

The work of Gastinel, Vaisman, and Dunoyer³ just cited is informative with regard to my lung density experiments for its description of organs in mice affected by tuberculin shock. The viscera of these animals, suffering severe shock after subcutaneous challenge, were found to be congested and hemorrhagic, and particularly the lungs were affected. Although challenges employed in the present lung density studies were insufficient to provoke overt symptoms, hemorrhagic lung congestion was a consistent pathologic characteristic of challenged, sensitized mice, and it would account physically for the lung density increases interpreted as reflecting delayed hypersensitive reactions.

Individually, the evidence from any single series of the experiments just discussed should be moderately convincing proof that mice and rats develop delayed hypersensitivity. Collectively, their evidence seems conclusive. If, then, mice and rats should be assumed so capable, they might be expected to exhibit the pathology of tuberculosis seen in the higher animals reactive to skin tests and thought closely related to this kind of allergy. Yet, it is common knowledge among those who use these animals in tuberculosis research that generally the microscopic and macroscopic tissue changes in tuberculous mice and rats are less extensive and less varied than in guinea pigs and rabbits, for example. Ordinarily, the pathology of these larger animals is described in terms of emergence of epithelioid cells, of giant cells, lymphocyte infiltration, caseation, fibrosis, and cavitation^{9, 19-22} whereas in mice and rats tuberculosis commonly evokes proliferative or necrotic-exudative lesions, macrophage rather than lymphocyte infiltration, and foam cells.^{14, 23, 24} Nevertheless, review of published data shows that, while not common, the changes seen in easily sensitized animals also have been observed in mice or rats and therefore that the latter animals probably need not be denied capability for developing tuberculin allergy on this basis.

Epithelioid cells have been observed in mice by Gray and Jennings⁴ and by Pierce and associates¹⁶ and in rats by Hehre and Freund.¹⁰ Smith and Hendrick¹³ found giant cells, multinucleated cells, epithelioid cells, and lymphocyte infiltration in infected rats. Giant cells and cells of the Langhans type are exhibited in photographs of sections of tuberculous rat lung published by Hehre and Freund.¹⁰ Although caseation is not common in mice and rats, it is not rare.^{3, 6, 14, 23, 24}

Formation of cavities in animals seems to be related to development of delayed hypersensitivity, if not dependent upon it. The recent intriguing experiments of Yamamura and co-workers^{25, 26} substantiate this, for they show that cavities can be induced to form in rabbits by appropriate immunologic procedures making specific use of tuberculin allergy. Cavitation in tuberculous mice apparently has been observed in only one laboratory. Levaditi, Vaisman, and Barrat²⁷ found 20 per cent of one series of chronically infected mice sacrificed 139 days after infection to have developed cavities in the pulmonary parenchyma. A later experiment involving three batches of sixty mice was less successful in this respect, since only two mice developed cavities. Observed microscopically, these cavities showed encapsulation by fibroblasts, large monocytes, and lymphocytes and had attained diameters of 1 to 2 mm. They were surrounded by heavy concentrations of tubercle bacilli. Levaditi and co-workers believe that the cavities were formed by a progressive softening of large tuberculous follicles followed by pus evacuation through bronchioles, and they provide photographic evidence to substantiate this belief. Apparently, since such cavities could not be induced at will in subsequent mouse experiments, the conditions under which they can develop in mice must be rather specific and, at present, are not well enough understood to control. Perhaps from the stimulus which this discovery may provide, vigorous attempts to induce cavitation in these animals and to learn under what particular set of conditions it can develop will be made in the future.

Although neither direct nor indirect evidence that mice and rats can develop delayed hypersensitivity is available in large quantities, enough has accumulated to be nearly conclusive. Special techniques have had to be developed to gather it, since sensitized mice and rats have responded to the traditional skin test as the higher animals do. Perhaps the passive transfer experiments of Wallace²⁸ offer an explanation for the ineffectiveness of skin tests in rats. Since Wallace was able to transfer delayed hypersensitivity from rats to guinea pigs, using rat leukocytes, it would seem that some reactive property of guinea pig skin is lacking in rat skin.

The original experiments reported in this article offer further direct evidence by employing a sensitive new technique. They have indicated that mice need not be infected with virulent tubercle bacilli to develop easily detectable sensitization, that sensitivity can be observed after intravenous injection of only a few hundredths of a milligram of tubercle bacilli, and that demonstrable active or passive immediate hypersensitivity is not associated with the delayed reactions observed.

SUMMARY

A new technique has been used to show that white mice can develop delayed hypersensitivity to tubercle bacilli. The technique consists of determining mouse lung densities after intravenous challenge with these bacilli, increased densities indicating the delayed allergic reaction. Aside from its high sensitivity, this method takes advantage of the observation that in sensitized mice undergoing such a reaction the major organs primarily affected are the lungs.

The reported experiments show that living, avirulent tubercle bacilli suspended in water-oil emulsions sensitize as effectively as similarly injected but buffer-suspended living, virulent bacilli. The delayed hypersensitivity reaction could be elicited with as little as 0.03 mg. of either virulent or avirulent bacilli. Active and passive sensitization experiments showed that the observed delayed lung reaction was not due to an anaphylactic (immediate) type of hypersensitivity to tuberculo-protein.

A short review of the literature concerned with the question of whether or not mice and rats can develop delayed hypersensitivity is presented, and from this the conclusion is reached that available evidence overwhelmingly favors the competence of these species to become allergic.

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