Reports

Delayed Hypersensitivity in Mice: Its Detection by Skin Tests and Its Passive Transfer

Abstract. Mice vaccinated intraperitoneally with a water-in-oil emulsion containing ovalbumin and avirulent tubercle bacilli developed strong immediate and delayed hypersensitivities, demonstrable by intracutaneous injection, to the ovalbumin. The two types of hypersensitivity could be differentiated by gross characteristics as well as by passive-transfer experiments with cells and serum from actively sensitized donors.

Recent work from several laboratories has challenged the long-held belief that mice cannot develop delayed hypersensitivity (*1, 2*). Unsuccessful attempts to evoke delayed-type skin reactions in presumably hypersensitized mice (*1, 3*) have caused slow acceptance of results from such experiments. Although reasons why these skin reactions have not been observed before now are not clear, the experiments reported below may contribute information toward eventual explanations as well as knowledge helpful to those using mice in experiments possibly influenced by delayed-type hypersensitivity reactions (*4*).

Twelve-week-old CF#1 white mice (Carworth Farms) were kept in small groups and supplied liberally with Rockland mouse diet and water. One set of 19 mice was injected intraperitoneally twice at weekly intervals with 0.1 ml of water-in-oil emulsion (*5*) containing 0.25 mg, moist weight, of living H37Ra strain tubercle bacilli and 0.25 mg of twice-crystallized ovalbumin (Nutritional Biochemicals Corp.). A second set of seven mice received similar injections of the emulsion alone. Two weeks after the second injection, all control and 15 test mice were challenged intracutaneously with 1 percent ovalbumin dissolved in physiologic sodium phosphate buffer at *p*H 7.4. This was done by anesthetizing each mouse with 1 mg of sodium secobarbital (Lilly) in 0.2 ml buffer, which was injected intraperitoneally, taping it to a glass plate so as to stretch the skin of the flank, clipping and shaving off the hair in this area, and injecting approximately 0.01 ml of ovalbumin solution just beneath the surface of the skin. This injection produced a bleb about 2 mm in diameter and 1 mm high which, in unsensitized mice, disappeared within about 30 minutes, leaving only a flat, blanched spot which usually persisted for at least 24 hours.

Reactions in actively sensitized mice were recorded in terms of induration diameter, relative skin thickening, and presence or absence of central necrosis. Twenty-four- and forty-eight-hour readings made in this experiment are shown in Table 1 and clearly differentiate test from control mice. The skin reactions resembled those of delayed hypersensitivity in guinea pigs mainly by the important common property of induration. Although spotty redness, apparently due to intracutaneous bleeding, also was common, there was no true erythema. Central necrosis was shallow, the affected area having an amber color and resembling wrinkled parchment. Preceding these apparent delayed-type reactions by several hours were strong immediate reactions peaking between 3 and 6 hours after challenge and then subsiding. They were characterized by edematous swelling as well as occasional petechiae.

Chase and others (*6*) have shown that delayed hypersensitivity can be transferred passively with lymphoid cells from hypersensitized donors but not with their antiserum which transfers only immediate hypersensitivity. Passive transfer of delayed hypersensitivity in mice has not been reported. An attempt at it seemed appropriate in enlarging upon the above observations.

Four mice of the sensitized group, saved from skin-testing to avoid possible desensitization, and five normal mice were used as cell and serum donors. Each donor was etherized lightly and then killed by bleeding from severed major armpit vessels, its blood being collected and pooled with that of other donors of a set. The clotted, pooled bloods from each set of donors were kept for 1 hour in a 37°C water bath for clot retraction, and then the serum was poured off and centrifuged free of blood cells at 500*g* for 15 minutes. One-tenth of a milliliter of serum was injected intraperitoneally into each of five normal recipient mice. Separate reversed passive cutaneous anaphylactic titrations in mice (*7*) showed 0.02 ml of 0.03125 percent test serum injected intradermally to be the lowest of a series of halving dilutions to react when 2 mg of ovalbumin was injected intravenously.

Immediately after a donor's blood had been collected, its rib cage was opened and its thymus gland and attached lymph nodes were removed. These tissues from each set of donors were pooled in 10 ml of Tyrode solution containing 0.0125 percent gelatin kept at 37°C and then homogenized in a medium-grind, large Ten Broeck tissue grinder by two slow, complete, twisting excursions of the plunger. The resulting homogenate was centrifuged at 500*g* for 15 minutes to sediment the cells, and most of the lightly turbid supernatant fluid was withdrawn and discarded, leaving 2.5 ml which was used to resuspend the cells for injection. Four-tenths of a milliliter of this suspension was injected intraperitoneally into each of five normal recipient mice within less than 1 hour after removal of the tissues from the first donor. Since in these preliminary experiments passively transferred activity was not meant to be equated with any particular type of cell and crude tissue homogenates were used, it is sufficient to state that each recipient was injected with 0.006 ml of packed tissue.

Recipients in the passive transfer experiment all were challenged intracutaneously with 0.01 ml of 1 percent ovalbumin, just as actively sensitized mice had been, serum recipients 24 hours and cell recipients 48 hours after passive transfer. Since both immediate and delayed types of reaction were expected, skin test sites were observed several

Table 1. Reactions in actively hypersensitized mice 24 and 48 hours after intracutaneous challenge with ovalbumin.

Group	Induration*	Skin thickening†	Central necrosis‡
	24 hours		
Control	0.1	0	0
Sensitized	5.3	2.5	9/15
	48 hours		
Control	0	0	0
Sensitized	3.9	2.0	9/15

* Millimeters mean diameter. † Times normal skin thickness. ‡ Proportion reacting.

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

times during the first hour, and then at 3, 4, 5, 6, 24, and 48 hours after challenge.

In no case did recipients of normal serum or cells react to ovalbumin challenge. Mice receiving serum from sensitized donors developed edematous reactions of maximum mean diameter (10.6 mm) 3 hours after challenge, often accompanied by petechiae. These reactions lasted, somewhat diminished, through the sixth hour and had disappeared when the next (24-hour) reading was made. They never showed induration or necrosis. Hypersensitized tissue recipients, on the other hand, showed no reactions until the 6-hour reading, when induration, but no edema, was commencing. Induration continued to develop through the 48th hour when mean induration diameter was 1.6 mm and all mice showed central necrosis.

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References and Notes

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4. This work was aided by National Science Foundation grant G4025.
5. Physiologic sodium phosphate buffer (*p*H 7.4) : *n*-hexadecane : Arlacel A (10 : 4 : 1). Arlacel A (mannide monooleate) was a gift of the Atlas Powder Company, Wilmington, Del. See J. Freund, *Advances in Tuberc. Research* 7, 130 (1956).
6. See the recent review on this subject by B. H. Waksman, *Progr. Allergy* 5, 349 (1958).
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23 March 1959

Paramyosin and Contraction of "Catch Muscles"

Abstract. The isotonic shortening of glycerol-extracted preparations of molluscan catch muscles is inhibited at *p*H's and ionic strengths at which extracted paramyosin crystallizes. The isometric tension development is hardly altered under the same conditions. Consequently, the "catch mechanism" is explained on the basis of the crystallization of paramyosin.

The unusual features of the mechanical responses and the metabolic activity of certain molluscan and annelid muscles led earlier investigators to postulate a "catch mechanism" (*1*) by means of which these muscles could remain contracted for long periods without a large expenditure of energy (*2*). These muscles can be brought into a condition which resembles a reversible rigor in isolated preparations also by the use of certain stimuli. Resistance to stretch is increased, and the muscle can sustain loads which exceed by several fold the maximal load which it can actively lift. The quick-release phenomenon is missing during this period (*3*). These muscles are also characterized by a periodicity revealed in small-angle x-ray diffraction (*4*) and electron optical studies (*5*). This unique fine structure has been attributed to the presence of paramyosin, a protein which, when extracted and viewed after drying and staining, has a great tendency to show periodic structures (*6*). Paramyosin has been obtained only from the so-called catch muscles, but its amino acid composition shows some similarities to that of proteins of the tropomyosin class (*7*) .

A connection between the presence of paramyosin and the "catch mechanism" is an intriguing possibility. In this report experiments are described which tend to support this view and which provide a model for the control of the elastic properties of "catch muscles" based on the behavior of extracted paramyosin.

For the preparation of paramyosin the adductor muscle of *Venus mercenaria* was blended for 1 minute in 0.1*M* KCl and washed three times in 20 volumes of 0.1*M* KCl. The residue was extracted in 3 volumes of 0.6*M* KCl, containing 0.04*M* *p*H 7.5 Tris buffer. To the extract 3 volumes of 95-percent ethanol was added. The precipitate was resuspended in 0.6*M* KCl containing 0.01*M* neutral phosphate buffer and dialyzed against 10 volumes of 0.6*M* KCl containing 0.01*M* neutral phosphate buffer. Paramyosin went into solution, while actomyosin, which is denatured by ethanol, remained insoluble, and was removed by centrifugation. Paramyosin was crystallized by dialyzing it against 6 volumes of 0.01*M* *p*H 6.0 phosphate buffer and redissolving in neutral 0.6*M* KCl. After recrystallization the protein was stored by lyophilizing it from 0.6*M* KCl solution.

For a second series of experiments, the anterior byssus retractor muscle of *Mytilus edulis* was prepared by removing the muscle, along with a piece of the shell, from the animal and stretching it slightly on a wooden frame. This unit was immersed in aerated sea water for 1 hour and then placed in a 50 percent (by volume) glycerol-water mixture at 0°C for 24 hours. These muscles were stored in 50 percent glycerol at −20°C for at least 2 weeks before use. Cell bundles 100 to 150 μ in diameter were stripped from the muscle for measurements of tension and shortening. Prior to these measurements, the bundles were

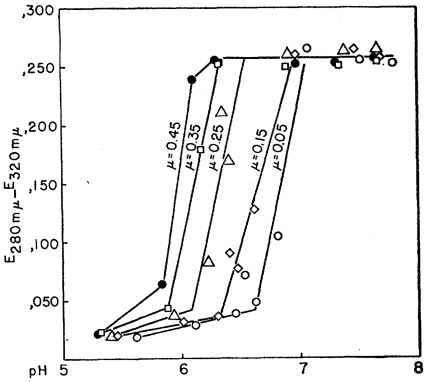

Fig. 1. Solubility of paramyosin. Paramyosin (0.9 mg/ml final concentration) was allowed to stand for 3 hours at room temperature. It was centrifuged, and the protein of the supernatant was measured by ΔO.D. 280 to 320.

equilibrated, first in 20 percent glycerol for ½ hour, and then for 15 minutes in an ATP-free solution of the desired ionic strength and *p*H. The fiber was finally transferred to the ATP-containing solution of identical *p*H and final ionic strength.

Crystallization is an outstanding property of paramyosin (*7, 8*). The protein forms needle shaped crystals—though with no faces and edges—in aqueous media which dissolve when the *p*H is changed by a relatively small amount. It was observed that a change of half a *p*H unit was sufficient to dissolve or reform the crystals (Fig. 1). The point of transition depended on the ionic strength, but stayed between *p*H 6 and 7.5 within the range studied (μ = 0.05 to 0.45), shifting toward the acid side with increasing ionic strength. Neither 0.01*M* $MgCl_2$ nor 0.01*M* $CaCl_2$, when added to the solution, had a measurable effect on the solubility of the paramyosin. Adenosine triphosphate (4m*M*) increased the solubility at lower ionic

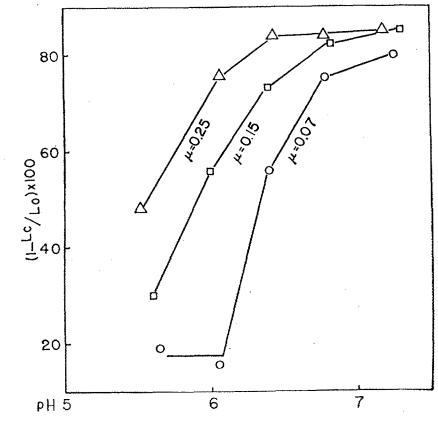

Fig. 2. Isotonic shortening of glycerinated preparations of anterior byssus retractor muscle of *Mytilus edulis*. Ordinate: *Lc,* contracted length; *Lo,* initial length.