SYSTEMIC DELAYED HYPERSENSITIVITY IN MICE

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Mice, like guinea pigs, develop acute anaphylaxis, and in both species humoral antibodies also can be responsible for a more protracted form of systemic shock, sometimes termed "protracted anaphylaxis". This is characterized in the mouse, as shown here, by a profound drop in temperature. In mice with simultaneously existing humoral antibody and cellular antibody hypersensitivites to an antigen, recovery from such hypothermia is delayed by several hours, an observation which had been thought in the past to indicate that hypothermia also is a characteristic of delayed hypersensitivity systemic reactions. But hypothermia was not evident in mice with delayed hypersensitivity uncomplicated by coexisting anaphylactic antibodies; this was discovered using the polysaccharide antigen dextran and in mice sensitized with entire avirulent tubercle bacilli.

The chemical natures of antigens used in these experiments were examined by destroying proteins with Pronase and polysaccharides with periodic acid oxidation. These studies revealed that the type of antigen responsible for dermal and systemic immediate and delayed reactions to ovalbumin, human serum albumin, and tuberculoprotein is protein, but that polysaccharide is the determinant in dextran responsible for delayed hypersensitivity to this substance (no immediate hypersensitivity developed to dextran). This report contains what apparently is the first description of a systemic delayed hypersensitivity reaction to a polysaccharide.

This abstract of about 250 words is approved as to form and content.

I recommend its publication.

Signed Alfred J. Crowle.

Instructor in charge of dissertation

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INTRODUCTION

Although delayed cutaneous hypersensitivity to many antigens has been demonstrated (3,4,5), the systemic delayed reaction most often has been studied using antigens extracted from microorganisms (1,13,23); few experiments have been done using purified protein antigens from nonbacterial sources (12,30), and none involving a polysaccharide have appeared. Nearly all have been done with guinea pigs.(1,4,12,13) Therefore, it seemed worthwhile to obtain data descriptive of delayed systemic reactions as they can be elicited in mice with purified nonbacterial proteins and to investigate such reactions as provoked by a polysaccharide, when this was discovered practicable.

HISTORY

The characteristic delayed allergic response of guinea pigs to the tubercle bacillus was first described by and has been named after Koch. (1) Classically, this delayed or cellular antibody hypersensitivity differs from immediate or humoral antibody hypersensitivity by two basic criteria: 1) delayed hypersensitivity cannot be transferred passively with serum but is instead transferred with lymphoid cells, whereas the immediate-type hypersensitivity can be transferred with serum but not cells, and 2) delayed hypersensitivity reactions generally are not seen for several hours after challenge with antigen, while immediate-type reactions develop rapidly.

Experimental induction of delayed hypersensitivity was difficult before the invention of Freund's complete adjuvant.(10) This adjuvant consists of a water-in-oil emulsion containing mycobacteria. By incorporating the antigen into it for injection into an animal one promotes induction of delayed hypersensitivity. Previously, delayed hypersensitivity was induced regularly only by infection with appropriate microorganisms, especially those which are acid-fast.(1) Once induced, cellular antibody hypersensitivity can be detected by skin tests or elicitation of specific systemic reactions.(3)

The course of a typical systemic tuberculin reaction has been described by Platt.(22) When a tuberculous guinea pig is challenged with a fatal dose of tuberculin its body temperature begins to fall in 2-3 hours and decreases linearally with time until death of the animal. This decline is associated with dullness, loss of appetite, disinclination to move, and elevation of the hair especially about the nape of

the neck. With deepening shock there is pronounced muscular weakness of the hind legs, revealed by the tendency for them to drag. The animal feels chilled and flacid to one's hand; upon being returned to its cage the animal will rock as if off balance and eventually becomes unable to stand. It undergoes intermittent convulsions, loses its reflexes, gasps irregularly, and gradually loses consciousness. Death occurs within 4-30 hours.

There have been conflicting and confusing reports of temperature changes accompanying tuberculin systemic reactions. Large doses of antigen injected into the highly sensitized animal elicit progressive hypothermia as described above.(22,26) But small doses of antigen administered to less sensitive animals cause fever.(26,30) Both fever and subsequent hypothermia are seen in appropriately challenged animals with intermediate sensitivity.(11,22,26) Temperature changes appear then to depend on the degree of sensitivity as well as on the challenging dose.

In a tuberculous animal various tissues can show a tuberculin response to a systemic challenge(1) and specific shock organs are not involved.(4) Autopsy reveals that the major sites of reaction are the lungs, which become hemorrhagic, and the walls of the stomach and intestines, which show hyperemia and/or hemorrhage.(4)

Systemic tuberculin reactions are similar among various species of mammals, but in the larger animal less antigen is required by body weight for induction of a fatal systemic reaction.(4)

Microorganisms other than tubercle bacilli have been used as antigens for inducing delayed hypersensitivity. For instance, tuberculin-type sensitivity has been induced with streptococci, Brucella melitensis,

Salmonella typhosa, Bordetella pertussis, and other bacterial organisms as well as fungal and viral antigens.(23) Systemic reactions, as well as skin reactions, have been seen during hypersensitivity to these and other microorganisms.(23,27) Purified proteins (5,12,30) and polysaccharides (8, 19, 25) also have been reported to induce delayed hypersensitivity, but systemic delayed reactions of tuberculin-type to these proteins and to polysaccharides have not been found in the literature.

Although systemic tuberculin reactions had been demonstrated without much difficulty in various experimental animals (1,4), early attempts to elicit systemic tuberculin reaction and skin reactivity in mice infected with tubercle bacilli failed.(14,15) But in 1952 Hart, et al, (16) reported that tuberculous mice showed systemic tuberculin reactions when tuberculin was given intravenously, although skin reactivity remained negative. After this, delayed systemic hypersensitivity to tubercle bacilli was confirmed in mice by other experimenters. (3,15,18,21) Delayed systemic reactions to Histoplasma capsulatum also have been reported in mice.(27) Classical delayed skin reactions of the tuberculin-type had not been elicited in mice to tuberculin or other antigens until 1959, at which time Crowle (5) demonstrated skin responses to intradermal injections of ovalbumin and tuberculoprotein.

In examining systemic delayed reactions, one must be aware of responses which may be confused with this reaction. Systemic anaphylaxis coexisting with systemic delayed hypersensitivity to the same antigen is probably the most perplexing and confusing.(13,17) Due to the sequence of reactions, the earlier appearing anaphylactic shock partially conceals the delayed systemic reaction.

Anaphylactic shock unaccompanied by the systemic delayed reaction is not confusing and is distinguished with ease, both grossly and

histologically.(4) But in the guinea pig symptoms of protracted systemic anaphylaxis resemble closely those of systemic reactions to tuberculin.(29) Protracted systemic anaphylaxis reaches a maximum 1-30 hours after challenge with a large quantity of antigen injected subcutaneously, intraperitoneally, or intramuscularly, and the reaction is accompanied by a temperature drop. This prolonged reaction and the temperature drop may lead to confusion with the systemic tuberculin reaction.

Jones-Mote hypersensitivity, induced by injecting minute amounts of antigen or antigen-antibody complexes formed with excess antibody, in some critical ways resembles tuberculin-type hypersensitivity.(4,24) It causes a delayed skin reaction upon intradermal injection of antigen and a mild delayed systemic reaction with fever upon systemic challenge, and it can be transferred passively with lymphoid cells but not with serum.(24) But this type allergy appears just a few days after vaccination only in the absence of circulating antibody, it is transient, being replaced by Arthus hypersensitivity, and it can be induced in guinea pigs without the aid of complete Freund adjuvant.(4) Interestingly, it has not been possible to induce in mice.(7)

As of the time when this research was undertaken, there was a need for further extension of knowledge of the characteristics of systemic tuberculin-type reactions, particularly in the relatively little studied mouse and with refined protein and polysaccharide antigens.

MATERIALS AND METHODS

ANIMALS: Six-week-old or older female CF-1 and CAF-1 mice were used. They were kept in small groups and fed commercial (Rockland) mouse pellets and water.

ANTIGENS: Ovalbumin (OVA), 3X crystallized (Nutritional Biochemical Corporation), human serum albumin, Fraction V (HSA) (Pentex, Inc.) and native dextran, 5-40 million molecular weight (Nutritional Biochemical Corporation) were the purified antigens used for sensitization and challenge.

H37Ra tubercle bacilli were grown on Kirchner medium made with sodium glutamate instead of asparagine. The organisms were stored lyophilized in the refrigerator until used. H37Ra protein antigen was precipitated from Seitz-filtered culture medium, on which the bacilli had been grown, by addition to the medium of 60 percent w/v ammonium sulfate. It was purified further by three precipitations with trichloroacetic acid, was dialyzed against distilled water, and finally was lyophilized and stored in the refrigerator.(3)

CHEMICAL MANIPULATION OF ANTIGENS: OVA, dextran and tuberculoprotein were digested with the proteolytic enzyme Pronase (20) and were oxidized with periodic acid.(9)

For Pronase digestion of proteins, 250 mg of the substrate were dissolved in 10 ml pH 7.4 physiologic phosphate buffer. Twenty-five mg of Pronase were added, and the mixture was incubated for 24 hours at 40°C. A batch of 500 mg of dextran was digested by the same technique. Control solutions of antigen also were incubated alone for 24 hours at

40°C, and Pronase alone was heated similarly. Merthiolate was added to each incubation mixture to a final concentration of 0.01 percent to repress possible bacterial contamination.

Oxidation of dextran by periodic acid (H_5IO_6) was accomplished by dissolving 100 mg of native dextran in 500 ml of pH 7.4 phosphate buffer and adding 500 ml of 0.2 M solution of periodic acid. After being incubated for 2 hours in the dark, the solution was dialyzed against distilled water at 4°C for 24 hours. The dialysate then was lyophilized and redissolved at a concentration of 10 mg dextran-equivalent in 0.2 ml phosphate buffer.

To prepare polysaccharide-free solutions of OVA and tuberculoprotein, 100 mg of each antigen were dissolved in 2 ml of pH 7.4 phosphate buffer and mixed with 2 ml of 0.2 M periodic acid in buffer. The solutions were incubated in the dark for 2 hours at room temperature, dialyzed against distilled water for 24 hours at 4°C and used undiluted in the experiments. Appropriate control solutions of the antigens were prepared without periodic acid oxidation.

The effectiveness of Pronase and periodic acid digestions were verified by coagulation, immunodiffusion and precipitation tests. For the coagulation test 1 ml of OVA treated with Pronase was heated to 87°C. Heat caused coagulation of the unaltered OVA; since Pronase-treated OVA did not coagulate, it was assumed to have been degraded. This finding was verified by immunodiffusion comparison of treated OVA with native OVA. In this test, mouse antiserum to OVA precipitated the native but not the treated OVA. The degrading activity of periodic acid was evident in that native dextran would form a fine white precipitate on addition of 95 percent ethanol, but periodic-degraded

dextran did not.

SENSITIZATION: Generally mice were hypersensitized with two subcutaneous injections of antigen in 0.1 ml volumes of water-in-oil (w/o) emulsion (3), given one week apart in alternate inguinal regions. Sensitization to tuberculoprotein followed an irregular schedule until sensitivity developed, because of technical difficulties. The antigen-w/o emulsion mixture consisted of 2 parts Myrevol[1], 3 parts n-hexadecane and 10 parts of pH 7.4 phosphate buffer. H37Ra tubercle bacilli were suspended thoroughly in the buffer with a fine-grind Ten Broeck grinder before emulsification. The other antigens dissolved readily in the buffer. OVA, HSA, and dextran were used at 0.25 mg per injection. H37Ra bacilli and tuberculoprotein varied from 0.1 mg to 5 mg per injection.

SKIN TESTS: Mice were skin tested with approximately 0.02 ml of a 0.1 or 1 percent solution of antigen in buffer.(3) Standard criteria for positivity (edema, induration, necrosis) were used to identify reactions.(3)

SYSTEMIC CHALLENGE: Mice usually were injected with 0.2 ml of antigen solution (concentration varying with the experiment) intravenously in the tail, but some groups also were challenged subcutaneously or intraperiteonally.

PASSIVE TRANSFER OF HYPERSENSITIVITY: Blood was collected from the axillary vessels of mice under light ether anesthesia. The pooled blood was allowed to clot at 4°C overnight. The serum was then poured

[1] A gift of Distillation Products Inc., Rochester, N.Y.

off and centrifuged free of blood cells. Varying volumes of serum were injected intravenously into the tail.

Immediately after a donor's blood had been collected, its spleen was removed. Pooled donor spleens were washed in heparinized Hank's solution (1 unit per ml), and mashed gently through a stainless steel wire screen, and the resultant dissociated cells collected. The cells were washed in more Hank's solution, centrifuged at 1500 rpm for 20 minutes, and then were resuspended to a concentration of 1 spleen-equivalent per 0.1 ml. One-tenth ml volume of this was injected intravenously, and the recipient mice were challenged immediately. In these cell suspensions, approximately 80 percent of the cells were alive by the eosin exclusion test.(28)

RECTAL TEMPERATURES: These were taken with a Tri R Electronic Thermometer with the thermocouple dipped in glycerol and inserted 15 mm into the rectum of the mouse.

AUTOPSIES: These were performed on most of the mice that died during the experiment and many were sacrificed by hyperextension of the neck for examination at the peak of the reaction. Particular attention was paid to the peritoneal cavity and the lungs.

RESULTS

TEMPERATURE STUDIES: Body temperature changes during systemic challenge with OVA, HSA, tuberculoprotein, and dextran were determined in mice 8-12 days after they had been skin tested. CAF-1 mice sensitized to HSA always showed good Arthus skin reactions but varied considerably relative to delayed skin reactions. On the other hand, CF-1 mice responded uniformly for both types of reaction to HSA.

Ten CAF-1 mice tested systemically with 3 mg of HSA developed hypothermia as shown in Figure 1 and charted by previously determined skin reactivity. The group of mice with a high percent showing delayed skin hypersensitivity took two hours longer to recover normal body temperature than the group with minimal delayed response. Controls (unsensitized) mice similarly challenged showed no significant temperature drop. CF-1 mice with both immediate and delayed skin hypersensitivity to OVA and HSA, upon systemic challenge, showed temperature variations similar to those in CAF-1 mice with both delayed and humoral-antibody hypersensitivities.

Passive transfer of the systemic delayed reaction with cells was unsuccessful, but the lag in temperature recovery was examined in mice with immediate hypersensitivity transferred passively with serum obtained from donors with strong immediate and delayed hypersensitivity. Donor serum was transferred 10 days after the donors had been skin-tested. Figures 2 and 3 show the temperature curves of mice which had received 0.2 ml of serum, results with recipients of 0.1 and 0.3 ml serum volumes being the same. These curves are compared with curves obtained for actively sensitized mice and nonsensitized control mice.

Figure 1. Mean body temperatures of HSA-sensitized CAF-1 mice challenged with 3 mg HSA, s.c., 10 days after skin test. Ten mice were used per group.

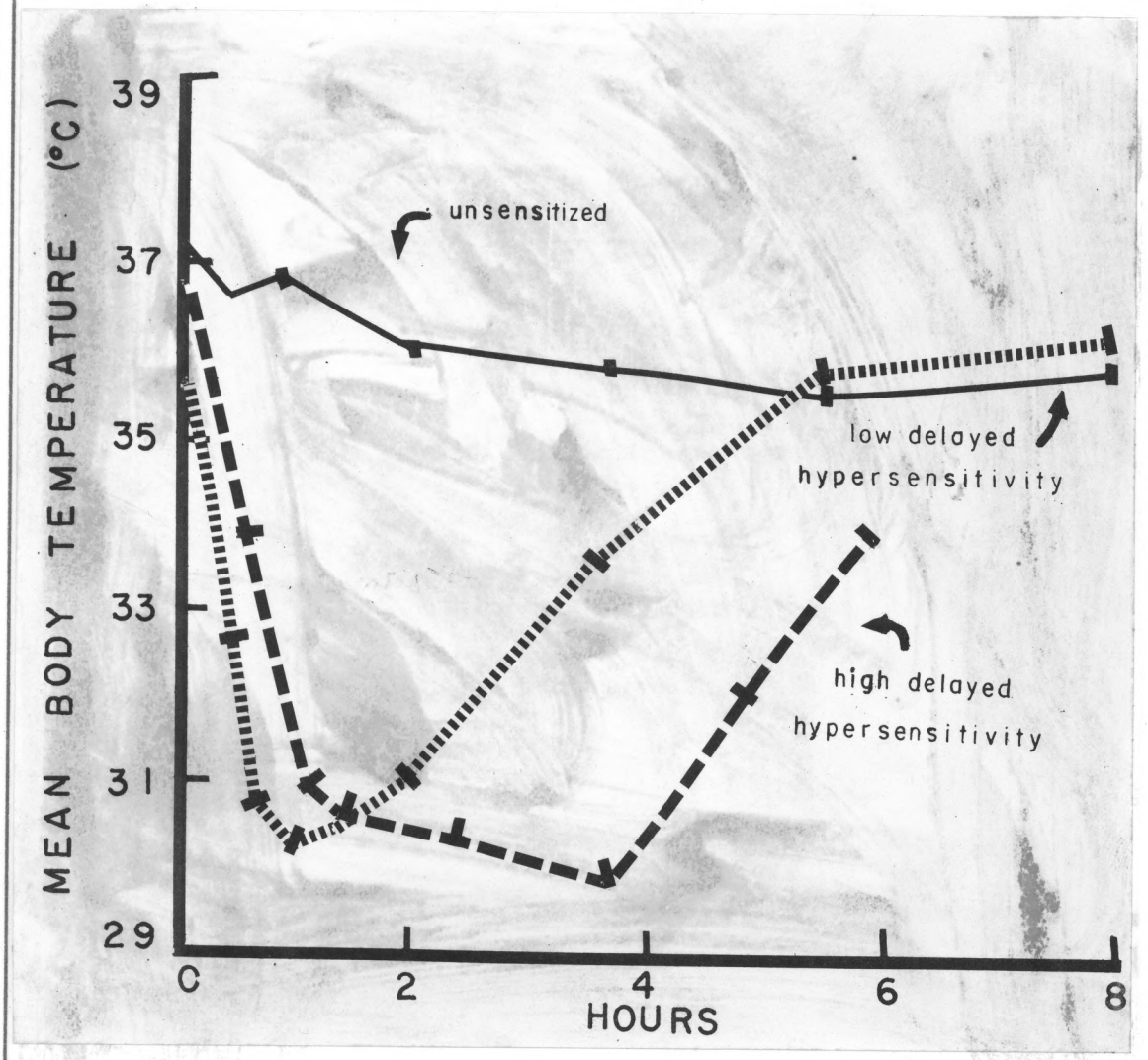


Figure 2. Mean body temperatures of CF_1 mice sensitized to OVA actively and passively with serum and challenged i.v. with 0.5 mg OVA, the actively sensitized mice 10 days after skin test. Ten mice were used per group.

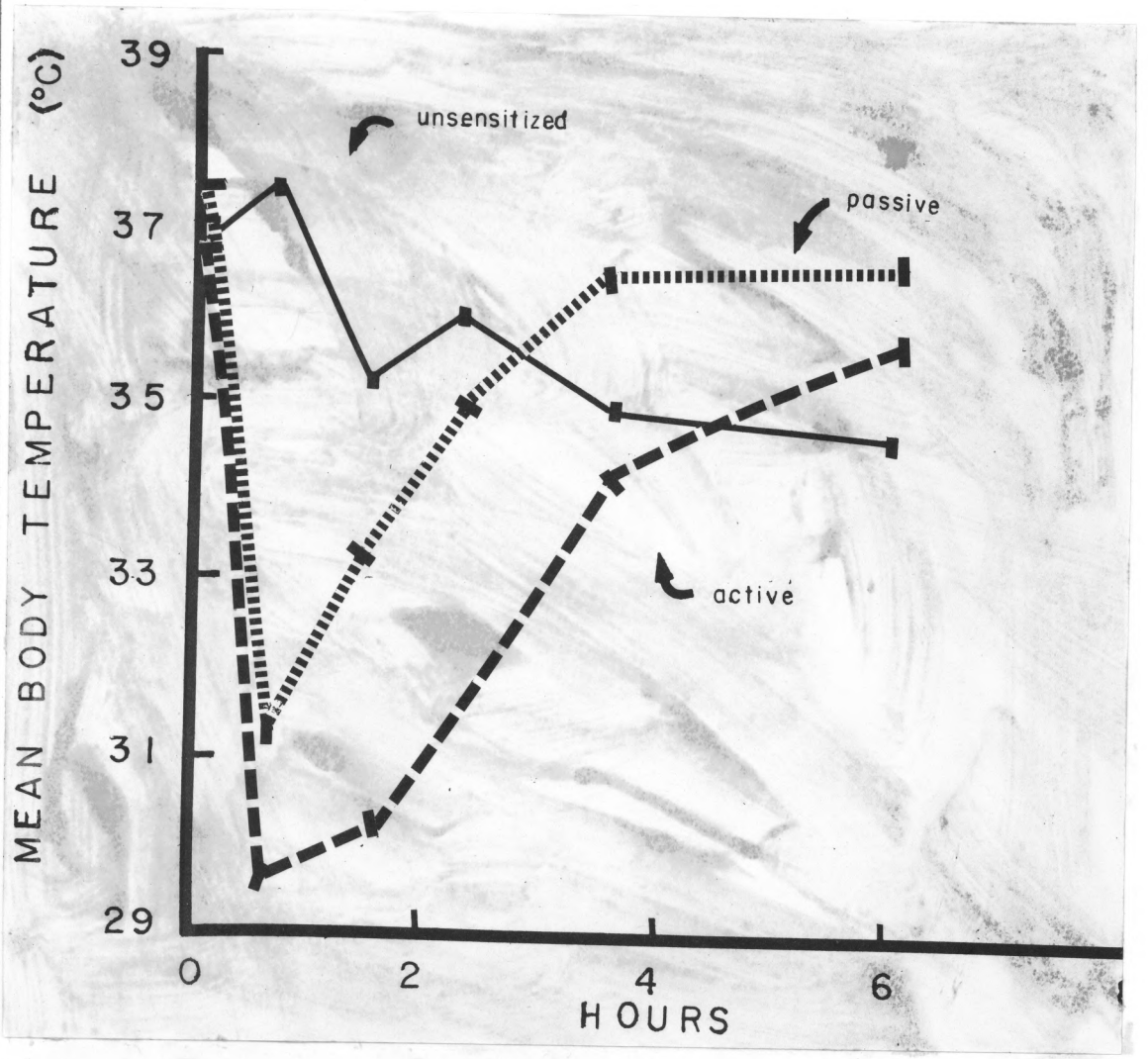


Figure 3. Mean body temperatures of CF_1 mice sensitized to HSA actively and passively with serum and challenged i.v. with 0.5 mg HSA, the actively sensitized mice 10 days after skin test. Ten mice were used per group.

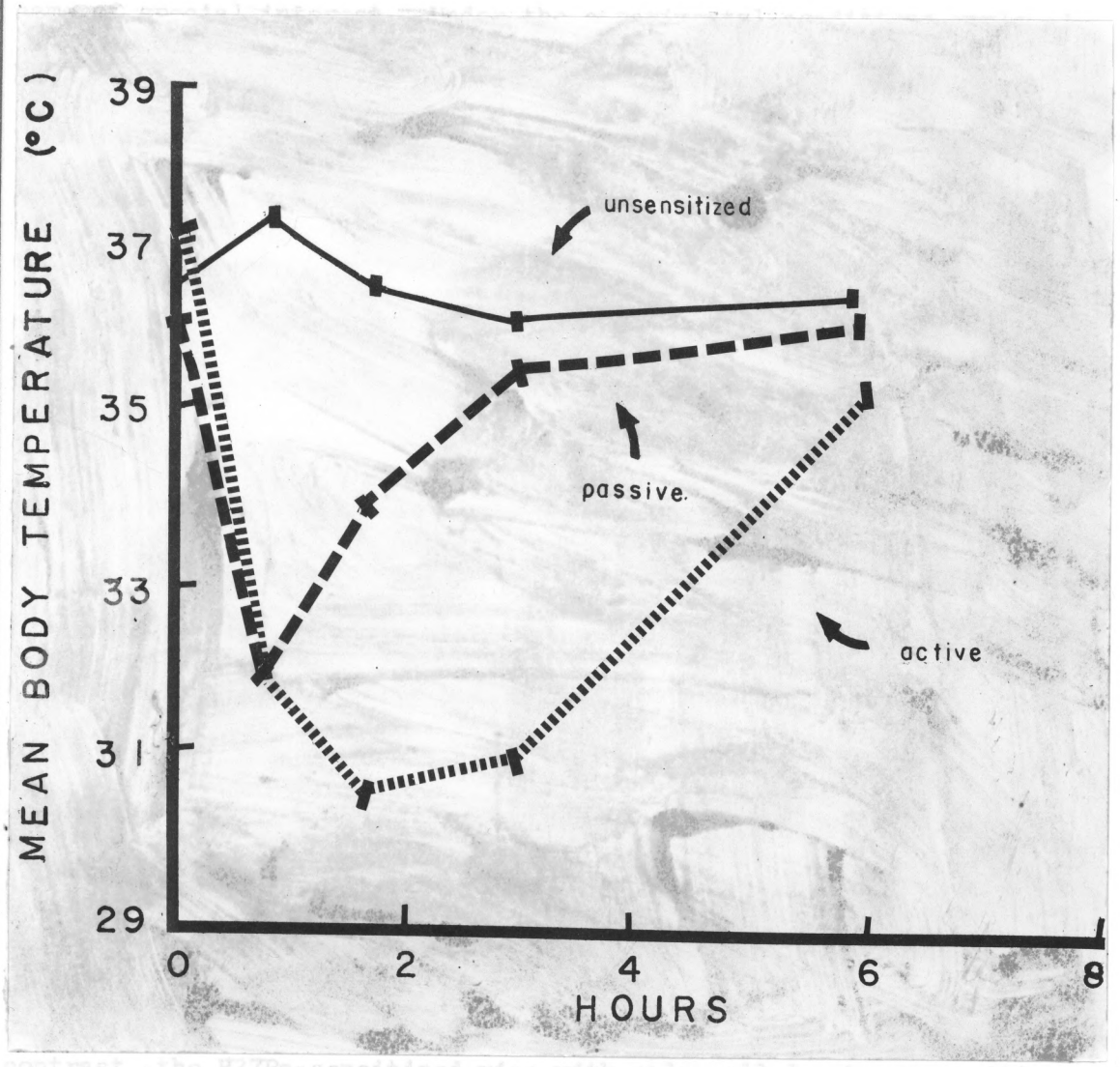
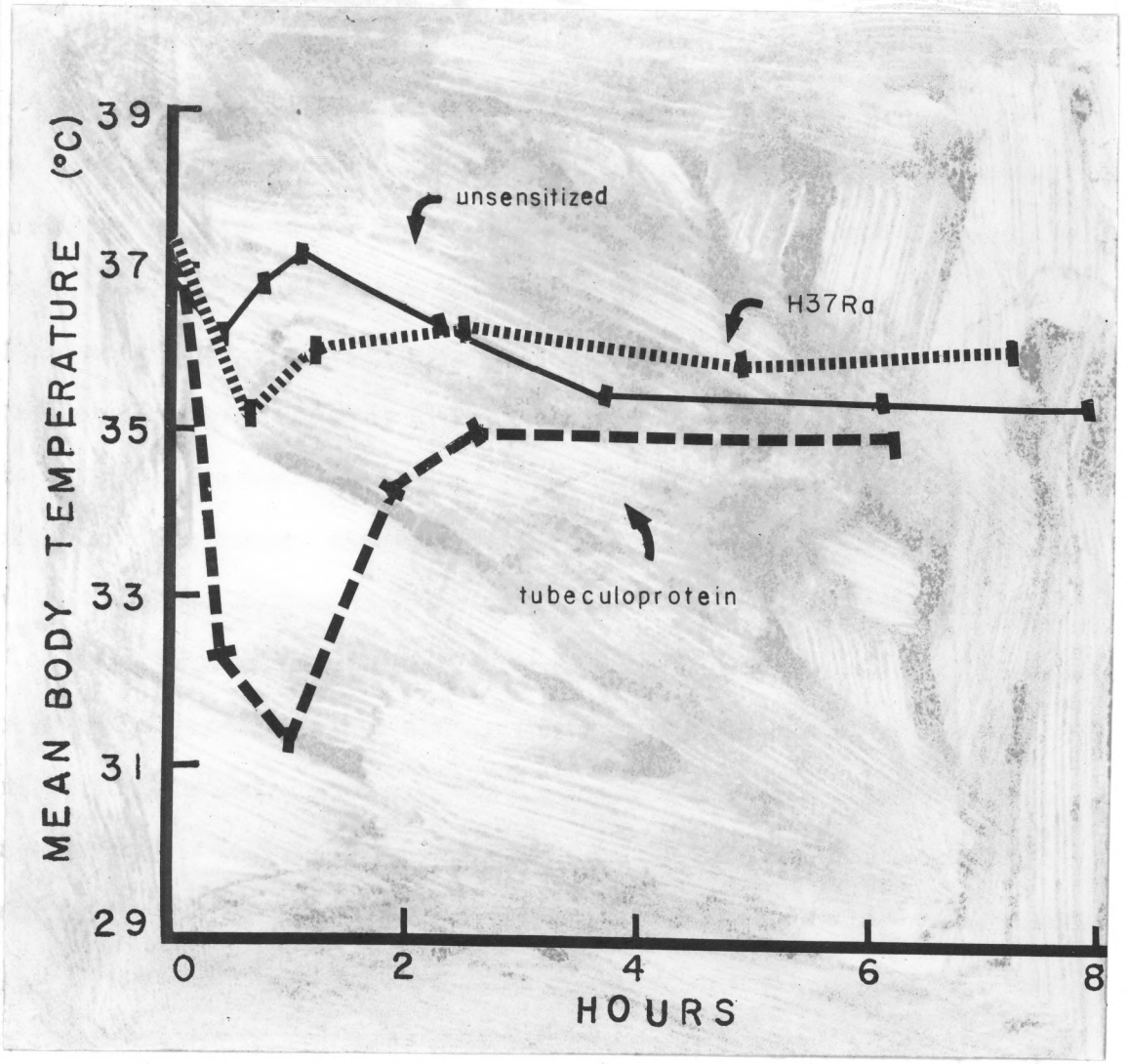


Figure 4. Comparison of mean body temperatures of tuberculoprotein and H37Ra-sensitized CF-1 mice challenged i.v. with 5 mg tuberculoprotein. Ten mice were used per group.



protein. As expected, unsensitized mice treated in a similar manner showed no reaction.

SYSTEMIC REACTION: Although hypothermia did not develop during systemic reactions in either dextran- or H37Ra-hypersensitive mice, a visible reaction did occur, and it was more noticeable in the dextran- and H37Ra-sensitized mice than in those sensitized to OVA or HSA. It was noticed first between 2 to 5 hours after challenge, and it would last sometimes up to 36 hours. Usually it had subsided in 24 hours. The mouse became lethargic, had a hunched appearance, and showed varying amounts of eye secretion. The fur was ruffled and the posterior portion of the animal dragged and was tucked under and forward. As the reaction progressed the animal became unwilling to move. Following this, the mice usually recovered but a few became comatose and died. Mice sensitized with OVA, HSA, or tuberculoprotein which developed antibodies along with delayed hypersensitivity showed the above reaction to a lesser degree. Among these, the reaction in the HSA system was the most pronounced, and that to OVA and tuberculoprotein reactions were the least noticeable.

The temperature of sensitized mice receiving dextran was taken at regular intervals and did not differ from the unsensitized controls receiving antigen. Unsensitized controls showed only slight reactions, never above + (see below), which disappeared by the sixth hour after challenge.

Table 1 shows a representative group of systemically challenged dextran-sensitized and -unsensitized mice. The degree of delayed systemic reaction is rated on an arbitrary scale from 0 to +++; 0 mice showed no reaction; + mice showed ruffled fur and slight hunching;

Table 1

DEGREE OF DELAYED SYSTEMIC REACTION FROM INDIVIDUAL DEXTRAN SENSITIZED MICE CHALLENGED I.V. WITH 10 MG OF NATIVE DEXTRAN, 10 DAYS AFTER SKIN TEST.

A +++ REACTION INDICATES MAXIMUM REACTION.

	Hours			
	3	6	12	24
Sensitized Mice	0	++	+++	+++
	0	++	+++	+++
	0	++	+++	+++
	0	++	++	+++
	0	+	++	+++
	0	+	+	+++
	0	+	+	+
Unsensitized Mice	0	++	0	0
	0	+	0	0
	0	+	0	0
	0	+	0	0
	0	+	0	0
	0	+	0	0

++ mice also had some eye secretions; +++ mice exhibited ruffled fur, hunched appearance, lethargy to the point of collapse, and copious eye secretion. As shown in the table nonspecific reactions in the unsensitized mice disappeared in 6 hours. Table 2 shows the same reaction criterion for the protein and tubercle bacillus antigens. Only the peak reactions are recorded.

Mouse delayed systemic reactions are said to be due to cellular-antibody hypersensitivity.(4) Therefore, an attempt was made to transfer this systemic reaction with lymphoid cells. Spleen cells of different concentrations from OVA- and dextran-sensitized donor mice were transferred to untreated recipients which were then challenged intravenously with homologous antigen. The OVA recipient mice elicited no signs of systemic reaction upon challenge. In the dextran system, four out of the seven recipient mice showed typical delayed reactions. These lasted up to 3 hours after the nonspecific reactions in unsensitized mice had subsided. This suggests that passive transfer of systemic delayed hypersensitivity may have been successful. Parenthetically, passive transfer of skin reactivity to dextran with cells has been easy.

STUDIES WITH CHEMICALLY MANIPULATED ANTIGENS: The chemical treatments carried out on OVA, tuberculoprotein, and the dextran with Pronase and periodic acid rule out the possibility of contaminants causing the delayed systemic reaction. This is illustrated by results of systemic challenge of unsensitized and sensitized mice with the treated antigen as shown in Table 3. Challenge antigens were either treated as described previously with Pronase or periodic acid or sham treated without Pronase or periodic acid. Results are recorded in proportions of reactors and types of reactions seen. These clearly indicated that protein is the

Table 2

DEGREE OF DELAYED SYSTEMIC REACTIONS OF INDIVIDUAL SENSITIZED MICE CHALLENGED WITH THE SPECIFIC ANTIGEN. READINGS WERE RECORDED FROM 12-24 HOURS AFTER CHALLENGE.

Antigens			
OVA	HSA	Tuberculo-protein	*H37Ra
+++	++	+++	+++
+++	+	++	+++
++	+	++	+++
++	+	++	++
+	0	+	++
+	0	+	++
		+	++
		0	++
		0	++

*Mice were sensitized with H37Ra and challenged with tuberculoprotein.

Table 3

SYSTEMIC CHALLENGE OF UNSENSITIZED AND SENSITIZED CF-1 MICE WITH THE SPECIFIC PRONASE AND PERIODATE TREATED ANTIGENS

[1] Mice sensitized with OVA.

[2] Mice sensitized with TBP.

[3] Mice sensitized with native dextran.

[4] "DRS" = delayed systemic reaction.

[5] "TBP" = tuberculoprotein.

[6] "Sham" = sham-treated without either Pronase (upper) or periodic acid (lower).

		Number responding/ number tested		
Challenge Material	Challenge Dose-mg	Unsensi-tized	Sensi-tized	Type Reaction
[1] OVA + Pronase	0.5	0/3	0/7	None
Sham OVA[6]	0.5	0/5	10/10	Anaphylaxis and DSR[4]
Pronase	-	0/5	0/5	None
OVA + Periodate	0.5	0/5	6/6	Anaphylaxis and DSR
Sham OVA	0.5	0/5	5/5	Anaphylaxis and DSR
[2] TBP[5] + Pronase	5	0/5	0/5	None
Sham TBP	5	0/5	5/5	Anaphylaxis and DSR
Pronase	-	0/5	0/5	None
TBP + Periodate	5	0/5	5/5	Anaphylaxis and DSR
Sham TBP	5	0/5	6/ 6	Anaphylaxis and DSR
[3] Dextran + Pronase	10	0/3	7/7	DSR
Sham Dextran	10	0/10	10/10	DSR
Pronase	-	0/5	0/5	None
Dextran + Periodate	10	0/10	0/10	None
Sham Dextran	10	0/10	8/8	DSR

essential antigen eliciting OVA and tuberculoprotein reactions and that a polysaccharide is responsible for dextran-elicited reactions. Similar results were obtained with skin tests rather than systemic tests as shown in Table 4.

AUTOPSIES: Autopsies done on mice that died of anaphylactic shock and on those sacrificed at the height of the delayed systemic reaction showed two distinct characteristics. 1) Mice that died of anaphylactic shock had darkening and hemorrhaging in the lower walls of the stomach. The small intestines were slightly erythematous and contained varying increased amounts of fluid. 2) Mice that experienced only delayed systemic reaction, for example to dextran, showed varying degrees of redness of the small intestines; the stomach was not affected. The reaction area was confined to the duodenum and jejunum which were void of chyme and collapsed. When mice which experienced both anaphylactic shock and delayed systemic reactions (e.g. those with OVA) were examined and were sacrificed during the delayed systemic reaction and after recovery from anaphylactic shock, only manifestations of the delayed systemic reaction were seen.

Table 4

SKIN TEST READINGS OF SENSITIZED AND UNSENSITIZED MICE WITH PRONASE-TREATED ANTIGENS

Challenge Material	number reacting per number tested			
	Sensitized		Unsensitized	
	3 hr	36 hr	3 hr	36 hr
OVA + Pronase[1]	0/10	0/10	0/5	0/5
Dextran + Pronase[2]	0/10	10/10	0/5	0/5

[1] OVA-sensitized mice

[2] Dextran-sensitized mice

DISCUSSION

The work presented here characterizes systemic delayed hypersensitivity in mice to purified protein antigens and a polysaccharide. The systemic reaction to tuberculoprotein, which has been studied earlier (3,4,11,22), also was further examined here.

The antigens used most often in delayed hypersensitivity studies have been tubercle bacilli and their proteins. Therefore, the term "delayed hypersensitivity" often is interchanged with the term "tuberculin hypersensitivity". Since the antigens of the tubercle bacillus may differ from other purified proteins and polysaccharides, they may evoke a different reaction. Hence the characteristics of tuberculin-type reactions may not be alike for all antigens. In this paper the term "tuberculin hypersensitivity" refers only to reactions to tubercle bacillus antigens.

The delayed systemic reaction is elicited in sensitized mice by systemic challenge with the specific antigen. The gross systemic reaction in mice becomes visible 2-6 hours after challenge and is characterized by a lethargic hunched appearance and ruffled fur which are accompanied by eye secretions. The mouse breathes shallowly and rapidly and later irregularly. This reaction can last as long as 36 hours, but usually it recedes within 24 hours after challenge.

Mice sensitized to native dextran showed the purest systemic delayed-type reaction, since it was uncomplicated by anaphylactic shock. Gross symptoms in dextran-elicited shock resemble those seen in mice with tuberculin systemic reactions, except that there is no progressive temperature drop.(4) Mice sensitized to OVA, HSA, and tuberculoprotein undergo the delayed-type reaction preceded and partially concealed by

anaphylactic shock. Body temperature changes have been used frequently to follow the course of both immediate and delayed systemic reactions in various species of animals.(2,3,11,22,30) Hypothermia developed reproducibly in mice with both immediate and delayed hypersensitivities. Although their hypothermia was prolonged in anaphylactically shocked mice with delayed systemic reactions, and not in mice with only immediate hypersensitivity, apparently it was not related directly to the delayed reaction, but rather was a prolongation of the previously developing drop due to anaphylactic shock. The principal evidence for this is that no hypothermia developed in mice reacting to dextran in which no complicating anaphylactic hypersensitivity coexisted.(8) These findings were confirmed in H37Ra-sensitized mice. Interestingly, hypothermia never developed as a result of any of these reactions.

The tuberculin shock results obtained by Crowle (3), in which it was possible to elicit systemic tuberculin reaction in mice, could not be duplicated using the same doses. The H37Ra and tuberculoprotein were used after three years of refrigeration. Skin tests and systemic reactions were looked for under the same conditions and doses as in the original experiments.(3) When reactions were not seen, larger sensitizing and challenging doses were used and the systemic reactions were re-examined. The mice sensitized with tuberculoprotein developed severe anaphylactic shock whereas the H37Ra mice showed only the delayed systemic reaction.

Because difficulty was encountered in repeating the tuberculin systemic results Crowle (3) reported earlier with H37Ra and tuberculoprotein, these antigens were re-examined. Crowle (6) set up experiments in which he sensitized mice with different amounts of H37Ra and tuber-

culoprotein, alone or in combination. Also tubercle bacilli, Erdman strain, which had been stored for three years in acetone, were used as a sensitizing agent. The results showed that the H37Ra and tuberculoprotein had lost much of their antigenicity at the doses used and the Erdman had lost its antigenicity altogether. Hence storage seems to have caused a loss of antigenicity of these tubercle antigens, thus explaining the difficulty discussed earlier in getting a systemic reaction to these antigens.

Although passive transfer of delayed hypersensitivity to OVA or dextran as detected by skin test can be effected readily with sensitized spleen cells, analogous transfer detected by systemic tests was unsuccessful for the OVA system and weak for that of dextran. Perhaps for transfer of delayed systemic hypersensitivity more sensitized cells need to be transferred than are necessary for transfer of the skin reactivity.

Treatments of the various antigens with Pronase (nonspecific proteolytic enzyme) and periodic acid (polysaccharide oxidant) indicated that the antigenic portion of OVA and tuberculoprotein is a protein while the dextran antigen is a polysaccharide. This is of special interest considering the current controversy over the potentiality of polysaccharide antigens to either elicit delayed hypersensitivity or to provoke delayed-type reactions.(1,23) The present observations apparently are the first obtained that a polysaccharide not only can induce delayed hypersensitivity, but also provoke a delayed systemic reaction.

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