tent of HGA-OP binding, the chemical bond formed between the two moieties presumably would be primarily of the non-ionic, coordinate (hydrogen bond and van der Waal's) type. For steric reasons, the tanning agent (HGA-OP) would probably act as a multifunctional agent to form such primarily coordinate inter-chain cross-links between adjacent collagen polypeptide chains(2). The net effect would be stabilization and "hardening" of the collagen lattice structure.

Summary. Binding of monomeric, unoxidized (HGA) and polymeric, autoxidized (HGA-OP) homogentisic acid solutions to hide powder collagen preparations has been studied. It has been shown that there is no active binding of HGA to collagen, whereas there is extensive binding of HGA-OP to collagen. Coupled with previously presented observations, it is suggested that HGA-OP can act as a potent tanning agent for collagen. It is further suggested that ochronosis and degenerative joint disease observed in patients with alcaptonuria may possibly be explained by the *in vivo* occurrence of such a phenomenon.

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Anaphylaxis in *Bordetella pertussis*-Treated Mice. IV. Schultz-Dale Reaction.* (26242)

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Injection of *Bordetella pertussis* cells into mice increases the susceptibility of these animals to fatal anaphylactic shock, whether the shock is induced by passive or active means(1). *B. pertussis* cells also increase susceptibility of mice to various agents and stresses such as histamine, serotonin, cold stress, x-rays, etc.(1). *B. pertussis* does not, however, increase susceptibility of the mouse skin to passive cutaneous anaphylaxis (PCA) (2). The mechanism by which *B. pertussis* produces the changes observed is not well understood, although *B. pertussis* may indirectly interfere with adrenal function(3). *B. pertussis* enhances antibody response to various antigens(4,5), and most likely increases the susceptibility of mice to active anaphylaxis by stimulating antibody response to sensitizing antigen. In spite of the adjuvant activity of *B. pertussis,* the relationship of this effect to the increased susceptibility to anaphylaxis produced in mice by these organisms has been questioned(4,5). Irrespective of the role that the adjuvant effect has on active sensitization, the increased susceptibility to passively induced anaphylaxis cannot be explained by the adjuvant effect of *B. pertussis,* since in this case it can be shown that *B. pertussis*-treated mice become highly sensitive to fatal anaphylaxis with amounts of antibody that do not sensitize normal mice to fatal shock(3,6). In this paper the effect of *B. pertussis* cells on specific active and passive sensitization of smooth muscle will be described.

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Materials and methods. Mice. Female mice of the CFW strain weighing from 14 to 18 g purchased from Carworth Farms, New

City, N. Y., were sensitized with crystalline egg albumin (Ea), and the uterus subsequently used for the Schultz-Dale test. *Antigen.* The Ea used was 5 times recrystallized egg albumin prepared in our laboratories by the method of Kekwick and Cannan as outlined by Kabat and Mayer(7). *Antibody.* Two pools of mouse peritoneal fluid were used, one containing 151 μg and the other 2001 μg of antibody nitrogen (Ab N) per ml. These fluids were prepared by a method previously described(8). Antibody determinations were made by the method of Gitlin(9). A pool of rabbit anti-Ea serum containing 481 μg of Ab N/ml was also employed in some experiments. *B. pertussis cells.* Smooth *B. pertussis* cells grown in the liquid medium similar to that of Verwey *et al.*(10) and killed by addition of 0.01% thimerosal (merthiolate) were employed. *Schultz-Dale reaction.* The Schultz-Dale reaction was carried out in the tissue bath at 37°C. The Tyrode's solution employed in the bath was of the following composition:

Sodium chloride	8	g
Potassium "	.2	g
Calcium "	.05	g
Magnesium "	.1	g
Sodium dihydrogen phosphate	.05	g
Sodium bicarbonate	1	g
Glucose	1	g
Distilled water	1000	cc

pH of this solution was 7.6

Usually after mixing the reagents in the order stated, the solution was kept at least overnight at room temperature before using. In freshly made Tyrode's solution, the uterine muscles did not, in many cases, relax sufficiently to avoid nonspecific, spontaneous contractions. The uterine horn, connected to a kymograph by means of a lever which pulled the muscle strip gently, was allowed to remain in the bath until no spontaneous contractions occurred. Repeated washings of over-active muscles with Tyrode's solution usually stopped nonspecific contractions. Once the muscle had completely relaxed, the antigen (Ea) was added in a concentration of 0.05 mg per ml of bath. The mice employed were killed by breaking the spinal cord at the neck region and uteri were removed and placed in Tyrode's solution. These muscles could be stored for at least 24 hr at 2-4°C without affecting their reactivity. Muscles that did not give a specific contraction when antigen was added were subsequently challenged with 0.05 μg of serotonin or 10 μg of acetylcholine per ml of bath to test their reactivity.

TABLE I. *In Vitro* Anaphylaxis with Uteri from Passively Sensitized Mice.

Source of antibody	μg anti-Ea Ab N/mouse	Results*
Rabbit	96	7/10†
"	9.6	0/10
Mouse	2	10/10

* All muscle strips that had negative Schultz-Dale reactions contracted with 10 μg of acetylcholine/ml of bath.

† Numerator = No. of muscles with positive Schultz-Dale. Denominator = Total No. of muscles tested.

Experimental results. Exp. 1. Passive sensitization with mouse and rabbit antibody. Each of 10 mice was passively sensitized with 2 μg of anti-Ea Ab N injected intraperitoneally and 6 hr later the uterine horns were removed and tested in the tissue bath for their ability to exhibit the Schultz-Dale reaction. In addition, 10 mice received 96 μg of rabbit anti-Ea Ab and 10 received 9.6 μg. The results presented in Table I show that all uterine strips of mice which received 2 μg of mouse Ab N gave a positive contraction while none of the strips from mice which received 9.6 μg of rabbit Ab N showed any sensitivity. Of those mice receiving 96 μg Ab N, only 7 out of 10 strips tested gave definite positive reactions; the remaining 3 were doubtful.

After this preliminary experiment the following experiment was performed to determine exactly how much mouse Ab N was required to produce the Schultz-Dale reaction and to show whether pre-treatment with *B. pertussis* cells affected this sensitivity.

Exp. 2. Quantity of mouse antibody required for Schultz-Dale reaction in normal and B. pertussis-treated mice. To groups of 5 mice each, various amounts of antibody were given 24 hr before removal of the uterus. For each antibody level, 2 groups of mice were used: one normal group and the

TABLE II. Sensitivity of Uteri from Normal and *B. pertussis*-Treated Mice Receiving Various Amounts of Mouse Antibody.

μg of anti-Ea Ab N/mouse	Results: Uteri from normal mice	Results: Uteri from *B. pertussis*-treated mice
.0625	0/5*	0/5
.125	3/5	2/5
.5	4/5	4/5
1.0	4/5	4/5
2.0	5/5	5/5
4.0	5/5	5/5
8.0	5/5	5/5

* See Table I.

other treated 4 days before injection of the antibody with approximately 2 billion *B. pertussis* cells given intraperitoneally (Table II). Five of 10 uteri from mice receiving as little as 0.125 μg of mouse Ab N gave a Schultz-Dale reaction and 8 uteri of 10 mice receiving 0.5 μg Ab N gave positive Schultz-Dale reactions. Larger amounts of Ab produced sensitization of nearly all the uteri. No difference was found in quantity of Ab required to sensitize the uteri of either *B. pertussis*-treated mice or normal mice. All the uterine strips which failed to exhibit the Schultz-Dale reaction were tested with 0.05 μg of serotonin per ml of bath and found to be active.

Exp. 3. Development of Schultz-Dale reaction in normal and B. pertussis-treated mice after active sensitization with 0.5 mg of egg albumin. Forty mice received 2 billion *B. pertussis* cells mixed with 0.5 mg of Ea. Groups of 10 *B. pertussis* and Ea-treated mice and 5 of those that had received Ea alone were killed at different intervals of time after sensitization. Uterine horns were removed and challenged in the tissue bath as before (Table III). Uteri from mice in Groups 1, 2, 3 and 4 were tested 5, 7, 8 and 10 days, respectively, after administration of the sensitizing dose. As controls the uterine horns of 4 normal unsensitized mice were included with each of the 4 groups. Uterine horns obtained 5 days after injection of the antigen alone or with *B. pertussis* did not contract. On the seventh day, however, 3 of 10 uterine horns were found to be sensitive in the *B. pertussis*-treated group while none in the group which received Ea alone responded to Ea. Eight days after the sensitizing dose, 9 of the 10 uterine strips of the *B. pertussis* groups were found to give the Schultz-Dale reaction while only 1 of the 5 Ea sensitized mice responded to Ea. On the tenth day, all uterine strips tested in either groups had developed sensitivity to Ea. None of the control strips from unsensitized mice contracted after addition of antigen.

Discussion. The results presented here again point out the importance of employing homologous antibody in study of anaphylactic reactions in mice and perhaps all animals. As has been previously noted, rabbit antibody is far less effective in sensitizing mice to both fatal and passive cutaneous anaphylaxis (2,6). The present results show that rabbit antibody is also inferior in sensitizing uterine muscle of mice than is mouse antibody.

It was of interest to find that *B. pertussis* cells do not affect the sensitivity of muscle strips of mice receiving the preformed antibody in passive sensitization experiments. These results, as well as those obtained with respect to PCA, indicate that *B. pertussis* cells do not affect directly the reactivity of tissues, but rather that they affect other physiological, pharmacological or metabolic functions of the mouse. In the case of active sensitization of mice, *B. pertussis* accelerated the appearance of sensitivity of the muscle. This observation can be explained by the known adjuvant action of *B. pertussis* cells.

Although the present results do not elucidate the mechanism by which *B. pertussis* increases the susceptibility of mice to passive anaphylaxis, they do indicate strongly that

TABLE III. Development of *In Vitro* Anaphylactic Sensitivity in Normal and *B. pertussis*-Treated Mice Actively Sensitized with Ea.

Time after sensitization, days	Results*: Normal sensitized	Results*: *B. pertussis*-treated	Results*: Normal unsensitized
5	0/5†	0/10	0/4
7	0/5	3/10	0/4
8	1/5	9/10	0/4
10	5/5	10/10	0/4

* All muscle strips that gave a negative Schultz-Dale reaction responded to 10 μg of acetylcholine per ml of bath.

† See Table I.

B. pertussis does not interfere with localization of antibodies in tissues and that it does not make the tissues hypersensitive to an antigen-antibody reaction. This has been previously suspected since it has been observed that, although there is a marked difference in amount of antibody needed to produce fatal anaphylaxis, there is hardly any observable difference in amount of antibody required to produce anaphylactic symptoms such as ruffling of the hair, forced respiration, slight cyanosis, lethargy, etc.(3).

Summary. Mouse antibody was found to be more effective than rabbit antibody in sensitizing the mouse uterus to the specific antigen. It was also found that *B. pertussis* cells do not affect the sensitization of the mouse uterus when the mouse is sensitized by passive means. When the active method of sensitization of mice was employed, however, *B. pertussis* accelerated the appearance of sensitivity of the uterus as demonstrated by the Schultz-Dale reaction.

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New Phages Active Against Staphylococci that Are Resistant to the Conventional Phage Set.* (26243)

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During studies on staphylococcal infections at Boston City Hospital, it was found that a large and probably increasing number of infections were caused by staphylococci that were insensitive to the phages generally used for typing, even at concentrations of 1000 x the routine test dilution (RTD)(1). A large proportion of these strains had been cultured from serious infections such as septicemias and pneumonias and they were usually resistant to several of the antibiotics in general use. The isolation and some of the properties of 5 new phages that were found to be useful in typing these "nontypable" (NT) strains is described in this paper. Some findings with 2 other new phages related to 80/81 are also included.

Isolation of New Phages. For this purpose 108 nontypable strains cultured from different sources and with a varying antibiotic sensitivity were selected. They were all cross-cultured by the method of Fisk(2). An overnight culture of each strain was flooded on plates of trypticase soy agar and the inoculum was allowed to dry. A drop of a broth culture of each of the other strains was then applied on the surface in a similar manner as in the bacteriophage typing. The plates were read after incubation at 30°C for 18 hours. Some (24 of 5040 combinations) of these cross-cultures showed evidence of lysis. The areas of good lysis (7 in all) were

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