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Neutralization of Specific Reagins in Monkeys Passively Sensitized by Cross-Reactive Allergy Sera. (28217)

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It is well recognized clinically that individuals with pollenosis due to grass often exhibit skin reactions when tested with pollens of related grasses. Patients with insect allergy usually are hypersensitive to several related species within a genus and, often to different genera within an order(1,2). Recently it was demonstrated(3,4) that sera from some individuals hypersensitive to the dust from castor bean pomace will passively sensitize the skin of monkeys to castor pollen and also to the pollen and seeds of other species and genera of the spurge (Euphorbiaceae) family.

In the past it was extremely difficult to study the mechanisms of human allergic cross-sensitization and allergic cross-reactions because it was thought to be necessary to utilize direct skin tests upon allergy patients or the Prausnitz-Küstner tests upon passively sensitized human volunteers(5). The problem of testing for cross-reacting allergies in passively sensitized human beings is also complicated by the phenomenon of "hyporesponsiveness" described by Bowman and Walzer(6) and confirmed by others(7). These workers have shown that, in human beings, wheals at passively sensitized sites produce an impairment in the subsequent responsiveness of such sites to restimulation. The hyporesponsiveness at a previously reacted site usually involves a tissue factor and an antibody factor in variable degree. The tissue factor represents a completely reversible change which diminishes rapidly and disappears within 4 weeks. The immunological change supposedly represents injury by the wheal to all of the reagins at a passively sensitized site; it is nonspecific and is not reversible.

Because of the domestic and world-wide importance of the production and processing of castor beans and of the widespread occurrence of severe allergy to castor beans and castor bean pomace, the U. S. Department of Agriculture encouraged additional research studies utilizing passively sensitized monkeys in an effort to elucidate the nature of allergic cross-reactions to castor bean pollen and pomace and to other plants of the spurge family. This project required our having access to a large population of castor-allergic individuals, and a population sufficiently large does not exist in the United States.

Panzani has been treating patients allergic to the dust from castor oil factories in the city and environs of Marseille, France. His first case was described in 1951, and the num-

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ber of cases treated and documented has increased steadily and now exceeds 500. Observations upon 102 cases were published in 1957 (8). Most of these patients were hypersensitive to other materials also, the most common being allergies to spondylocladium mold and to house dust. Allergy to spondylocladium seems to be associated with allergy to dust from the castor oil pomace, and many of the patients appear to benefit from hypersensitization with spondylocladium and house dust extracts. The mold is present on potatoes in this region, and may get into the castor pomace by way of the cloth bags used for transporting both potatoes and pomace.

In this report we discuss clinical and laboratory studies upon the blood sera from a relatively large population of individuals with clinical histories of hypersensitivity to the dust from castor oil factories and from fertilizers containing castor bean pomace.

Experimental. Case histories were taken, skin tests conducted, treatments administered, and samples of sera drawn and lyophilized by Panzani. The lyophilized sera were shipped air freight from Marseille, France, to Albany, Calif., where the passive sensitization studies were conducted by Layton and co-workers.

The partially purified castor bean seed protein, arbitrarily coded "CB-S.R.I.," was prepared by repeated alcohol precipitation of a hot water extract of castor bean seed flour as previously described(9). The castor bean pollen extract was prepared from defatted pure castor bean pollen.

Twenty *Macaca irus,* 2 *Macaca mulatta* (rhesus), and 1 *Macaca speciosus* (stump tail) monkeys were used as recipients for reaginic human sera in the passive sensitization tests reported here.

Sixty samples of human sera were examined for skin sensitizing antibodies by passive tests in 6 *Macaca irus* monkeys. Twenty-six of these samples gave 1+ to 6+ reactions when the challenge was made by intravenous (i.v.) injection of partially purified castor bean seed protein. Thirty-four sera did not give significant reactions with the partially purified protein although the donors of these sera had shown some degree of skin reaction to a crude aqueous extract of castor beans. It is probable that certain castor allergens were lost during our purification procedure. The 26 sera highly reactive to CB-S.R.I. were examined to determine the thermal stability of the skin sensitizing antibodies. One-half ml samples of each of the reconstituted sera were heated at 56°C for 4 hours. Aliquots were withdrawn after 30 minutes, 1, 2, 3, and 4 hours. The heated sera were tested for skin-sensitizing antibodies by the monkey passive cutaneous anaphylaxis (P.C.A.) test. Certain of the heated sera contained traces of skin-sensitizing antibodies after ½ hour at 56°C, but none were active after one hour at 56°C. These results are characteristic for what are commonly designated reaginic antibodies. In another test it was shown that the skin-sensitizing human reaginic antibodies persist in the monkeys' skin for at least 2 weeks after sensitization.

Procedure. The *Macaca irus* monkeys were passively sensitized by intradermal injection of 0.05 ml aliquots of each of the 26 reaginic sera described above. From 48 to 76 hours after the sensitizing injection, each of the monkeys was injected intravenously with 4 ml of 0.5% Evans Blue dye and was challenged (i.v.) with a saline solution containing 10 mg of the lyophilized extract of pure castor bean pollen. Approximately one hour after the pollen injection the animal was photographed, then 5 mg of partially purified castor bean seed protein was injected (i.v.). Approximately 15 minutes later the animal was again photographed. Comparison of the two sets of photographs showed the differential reactions to the pollen and to the seed allergens. Examples are shown in Fig. 1.

The rhesus monkeys were sensitized in sites on the face, abdomen, arms, thighs and back of the legs with the sera which were reactive with ricinus pollen. The *M. speciosus* monkey was sensitized with these same sera in the same injection pattern as for the rhesus.

Neutralization of allergy reagins specific to ricinus pollen. Twenty-four hours after she was sensitized, the stump-tail monkey was given an (i.v.) injection of saline containing 20 mg of the solids extracted from pure ricinus pollen. No dye was used with the neutralizing (or "desensitizing") injections. The lesions elicited by the pollen were visible in 4 sites and were faintly visible in the remainder.

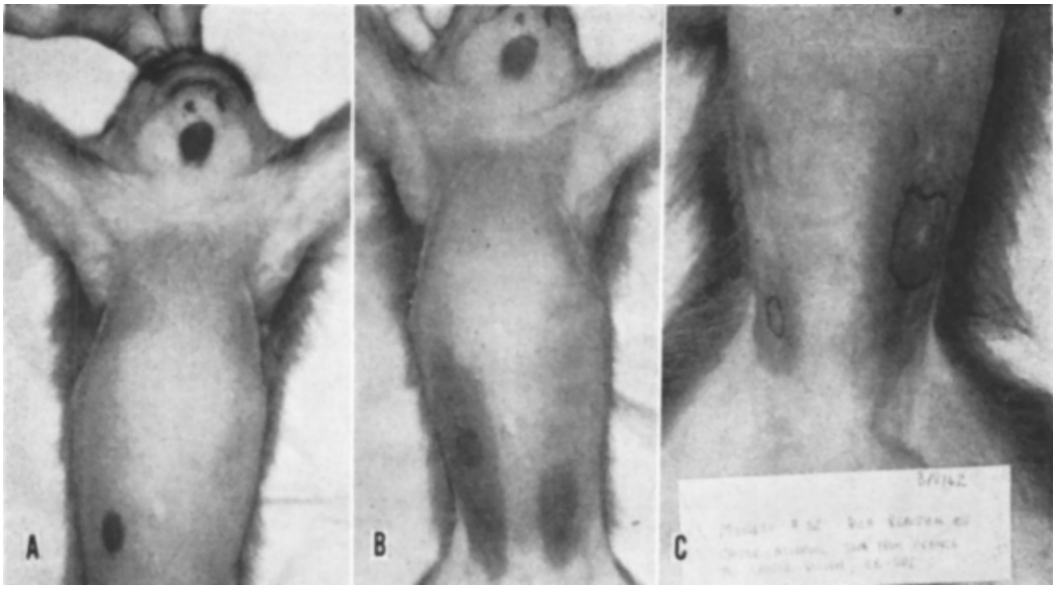

FIG. 1. A and B: Differential cross-reactions to pollen and seed of *Ricinus communis*. This monkey was sensitized with sera from human beings who are allergic to castor bean dust. Seventy-six hr after sensitization, the animal was challenged (i.v.) with 10 mg of castor pollen extractives. One hr later photograph A was made. The monkey was then challenged (i.v.) with 5 mg of castor bean seed protein CB-S.R.I. and 15 min later was photographed again (1B). The original lesion to pollen was outlined with a pencil. No change in chin site. C: Monkey sensitized in the same manner with other sera was treated in same manner as animal in A and B. The lesions resulting from the pollen challenge, outlined with a pencil, are shown within the larger areas reacting to seed proteins.

Forty-eight hours after the first pollen-desensitizing injection the monkey was given 5 ml of 0.5% Evans Blue dye; this was followed 15 minutes later by 20 mg of castor pollen extractives; there was no reaction to pollen. After half an hour the monkey was photographed. She was then given (i.v.) the extract from 5 mg of *Spondylocladium atrovirens*. Two face sites showed 1+ and 2+ reactions respectively, and when no further reactions to the mold could be detected the animal was again photographed (Fig. 2A). She was then given an (i.v.) injection of saline containing 10 mg of castor seed protein CB-S.R.I. Within 3 minutes all of the face sites and those of the abdominal sites which were to react had reacted, and maximal color had developed within 5 minutes. Fifteen minutes later the animal was again photographed (Fig. 2B).

The rhesus monkeys treated in the same manner as the stump-tail monkey gave comparable reactions.

Another monkey was first challenged with castor bean protein. This treatment desensitized the monkey to subseqeunt challenge with castor pollen extract. This result indicated that the pollen component(s) to which the individuals were hypersensitive occurred in the seed also. The patients from whom the sera were obtained probably were originally sensitized by contact with seed proteins rather than castor pollen.

Results and discussion. Challenges with the castor pollen extract elicited 2+ to 4+ reactions (Fig. 1A) in sites sensitized with sera from 6 of the 26 French patients selected for study. By comparing Fig. 1A with 1B, one easily can see differential reactions to the pollen and the seed allergens. In Fig. 1C (corresponding to 1B), we indicated the original reaction to pollen by outlining the pollen reaction with a pencil; all of the encircled lesions (color) were elicited by reactions to the subsequent (i.v.) challenge with seed protein. Additional reactions to pollen and to seed occurred upon the back of the legs, but these lesions are not shown. Lesions resulting from pollen reaction were confined to small areas and the edges of these lesions were sharply defined. The subsequent reactions to

TABLE I. Results of Clinical Tests upon Patients And Passive Sensitization Tests in Monkeys.

	Skin test reactions in patients		Passive reaction in monkeys	
	Castor bean		Castor bean	
Patient No.	Pollen extract	Seed extract	Pollen extract	Purified seed protein
1	2+	2+	4+	4+
2	2+	4+	4+	6+
3	2+	3+	2+	4+
4	2+	3+	2+	4+
5	3+	4+	3+	4+
6	2+	3+	2+	4+
7	2+	3+	0	2+
8	3+	4+	0	4+
9	2+	4+	0	2+
10	2+	2+	0	4+
11	2+	2+	0	4+
12	3+	4+	0	4+
13	3+	4+	0	4+
14	2+	3+	0	2+
15	3+	4+	0	4+
16	No case history		0	6+
17	3+	4+	0	4+
18	3+	4+	0	4+
19	2+	4+	0	4+
20	2+	3+	0	4+
21	4+	4+	0	1+
22	2+	2+	0	3+
23	2+	3+	0	4+
24	2+	2+	0	4+
25	1+	2+	0	4+
26	2+	2+	0	1+

seed allergens involved more sites, were more diffuse, and extended over much larger areas (except for the chin site of the animal in Fig. 1A and B).

The desensitizing injection of castor pollen extract given the stump-tail monkey 24 hours after sensitization appeared to have exhausted all sites of their anti-pollen reagins. This was evidenced by the failure of the second challenge with pollen to give any P.C.A. reaction whatsoever. Failure to react to the second challenge with castor pollen cannot have been the result of tissue refractoriness or histamine depletion, since 2 weak reactions were elicited by spondylocladium, and several very strong reactions were subsequently obtained with the seed proteins (Fig. 2A and B). Certain of the sites appeared to give relatively weakened reactions to the subsequent challenge with castor seed protein. Such weakened reactions to seed protein after neutralization of the anti-pollen reagins may have been the result of tissue hyporesponsiveness similar to that observed in human beings(6), or of relatively high serum titers of reagins to components common to pollen and seed, and corresponding lesser serum titers of reagins to the remaining allergens characteristic of the seed alone, *i.e.*, much less total antibody was left to react with the seed proteins. There appears to be no correlation between the intensity of the reaction to castor bean seed and that of the reaction to the pollen, but we have never observed, either clinically or experimentally, a case of hypersensitivity to the pollen without a corresponding reaction to the seed protein. Clinical and experimental data are presented in Table I. In the sera which did react with the pollen there were antibodies which either did not react with the pollen or which were only partially reactive to pollen and underwent additional specific reaction with allergens characteristic of the seed alone. This is clearly shown in Fig. 1 and 2.

Multiple allergies to seed and pollen are indicated by the experiment illustrated in Fig. 2A and B wherein the pollen-sensitive sites were completely and exhaustively desensitized to pollen while strongly positive reactions could yet be obtained in the same sites with the seed proteins.

These patients exhibited positive skin reactions to castor pollen extract, but only 6 gave significant serum reagin reactions to pollen in the passive tests. This discrepancy may be explained by the fact that serum levels of reaginic antibodies vary from time to time in an individual, while the skin sensitivity remains relatively constant for long periods of time after exposure to allergens. We have observed the phenomenon of serum reagin attenuation in all of the American cases of castor bean allergy that we have studied. Sera from individuals actively suffering from allergy due to exposure to castor pomace dust showed high titers of reagins to the seed, but sera drawn from these same individuals 11 months after their last known allergic attacks gave weakly positive or even negative passive anaphylaxis tests in monkeys. Subsequent respiratory exposure to castor dust elicited an immediate increase in the serum reagin titers as determined by the passive sensitization tests in macaque monkeys.

Several of the French patients were receiving palliative treatments with corticoids, anti-

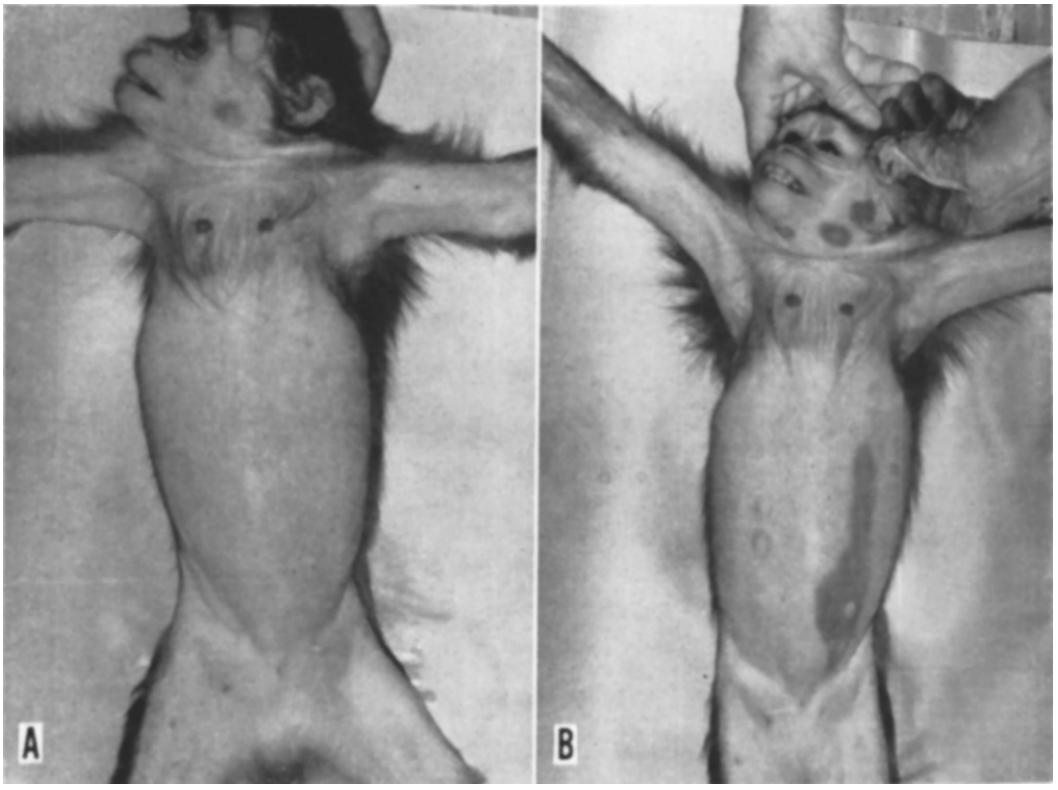

FIG. 2. Effect of prior neutralization of reagins specific for castor pollen in *M. speciosus*. This monkey was given a massive injection of castor pollen extract 24 hr after sensitization. A: Seventy-two hr after sensitization (48 hr after first pollen injection) the monkey was given Evans Blue (i.v.) and challenged with a second massive dose of castor pollen extract. There was no reaction. She was then challenged with spondylocladium mold extract and gave faint reactions on both cheeks. B: Subsequent challenge with seed protein of castor bean elicited reactions in sites originally sensitive to both castor pollen and seed but now desensitized to pollen.

histaminics and hyposensitization injections for house dust and spondylocladium mold. It is possible that these treatments, especially with the corticoids, may have affected the reagin level of the sera. House dust, if collected in Marseille, might be expected to contain at least traces of castor bean antigens since factory dust and fertilizer cause castor allergy all over the city and neighboring countryside(8)

A similar study of allergy to castor pollen among 13 castor-allergic American castor oil factory workers indicated that 9 of these were allergic to the pollen also, in contrast with the 6 of 26 castor-allergic French patients. The higher incidence of P.C.A. reaction to castor pollen among the American castor workmen (if a real difference) may be attributable to their close contact with the dry beans and solvent-extracted pomace. Castor beans used by the American factory were mechanically harvested and contained as much as 7½ to 10% trash, much of it pollen and female blossoms. Castor beans used by the French factories are hand picked and cleaned, with less than 1½% trash, most of this being the fibrous capsules (burs). All of the American cases were in California where castor beans are grown commercially, and other Euphorbiaceae occur as ornamentals and as weeds(3, 4). Castor bean plants are not ordinarily grown in France.

Summary. Blood sera from 60 allergy patients in Marseille, France, were examined by the reagin passive transfer test in macaque monkeys. Twenty-six of the sera were highly reactive with partially purified castor bean seed protein. Six of the reactive sera were

also highly reactive with castor pollen extract. By utilization of the phenomenon of reagin neutralization (specific desensitization) with castor pollen extract, sites passively sensitized with these 6 sera were rendered non-reactive to the pollen allergens; the pollen-desensitized sites were still highly reactive to the seed protein. The results appear to support the conclusion that allergy to castor bean pomace may be hypersensitivity to one or more of several components of the seed, pollen, or female blossoms.

The exchange of materials was approved by the U. S. Department of Agriculture and the U. S. Public Health Service. The U. S. Customs Service approved entry of the sera, duty free, for research purposes. William G. Murray performed the photographic services.

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Altered Mesothelial Permeability in the Guinea Pig Following Bilateral Vagotomy.* (28218)

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The acute hemorrhagic pulmonary edema observed after bilateral vagotomy in certain species is probably part of a generalized edema involving many tissues(1). Mechanisms proposed for the lung edema(2) can be considered under 2 headings: (a) increased filtration pressure at the pulmonary capillary-alveolar barrier, and (b) increased permeability of the membranes making up the capillary and alveolar walls. This study subjected the latter proposal to analysis.

Methods used to study the permeability of vascular walls are usually indirect and only semi-quantitative. A technique has been developed in this laboratory(3,4) for measuring *in vitro* the permeability of rabbit mesentery. These studies emphasized similarities between the permeability characteristics of capillary walls and of this mesothelial membrane. The method was modified slightly to determine the influence of vagotomy, alone and in combination with other treatments, on the permeability of mesentery excised from guinea pigs. Quantitative data are presented here to show that vagotomy does influence mesentery permeability.

Methods. Guinea pigs, 400 to 600 g, anesthetized with ether were subjected to midcervical bilateral vagotomy or a sham operation. Sham-operated animals were sacrificed at appropriate times by a blow on the back of the head. Immediately after sacrifice or death due to pulmonary edema, a portion of mesentery free of macroscopically visible blood vessels and fat was excised and mounted in a diffusion cell free of hydrostatic or osmotic gradients(5). The ions Rb^{86} and p^{32}-orthophosphate migrate independently across the mesentery apparently by passive diffusion(3, 4). Krebs-Ringer bicarbonate (pH 7.4) with trace amounts of the radioisotopes made up the superfusion solution. Tracer substances passing from the superfusate (source solution)

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